NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF TRIM® VX

IN WISTAR HAN [Crl:WI (Han)] RATS

AND B6C3F1/N MICE

(INHALATION STUDIES)

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

November 2016

NTP TR 591

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to the NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's carcinogenic potential.

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CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

K.R. Ryan, Ph.D., Study Scientist
R.A. Herbert, D.V.M., Ph.D., Co-Study Pathologist
K.S. Janardhan, BVSc, MVSc, Ph.D., Co-Study Pathologist
ILS, Inc.
C.R. Blystone, Ph.D.
M.C. Cora, D.V.M.
P.M. Foster, Ph.D.
M.J. Hooth, Ph.D.
A.P. King-Herbert, D.V.M.
G.E. Kissling, Ph.D.
D.E. Malarkey, D.V.M., Ph.D.
D.L. Morgan, Ph.D.
S.L. Smith-Roe, Ph.D.
M.D. Stout, Ph.D.
G.S. Travlos, D.V.M.
M.K. Vallant, B.S., M.T.
S. Waidyanatha, Ph.D.
N.J. Walker, Ph.D.
K.L. Witt, M.S.

Battelle Toxicology Northwest
Conducted studies and evaluated pathology findings

J.A. Dill, Ph.D., Principal Investigator
S.L. Grumbein, D.V.M., Ph.D.
B.K. Hayden
K.M. Patton, D.V.M., Ph.D.
M.J. Ryan, D.V.M., Ph.D.
L.M. Staska, D.V.M., Ph.D.

Experimental Pathology Laboratories, Inc.
Provided pathology review

M.H. Hamlin, II, D.V.M., Principal Investigator
T. Crabbs, D.V.M.
M.M. Gruebbel, D.V.M., Ph.D.

Dynamac Corporation
Prepared quality assessment audits

S. Brecher, Ph.D., Principal Investigator
S. Iyer, B.S.
V.S. Tharakan, D.V.M.

NTP Pathology Working Group
Evaluated slides and contributed to pathology report on 2-year rats (February 20, 2014)

G.D. Hill, D.V.M., Ph.D., Coordinator
ILS, Inc.
M.F. Cesta, D.V.M., Ph.D.
National Toxicology Program
S.A. Elmore, D.V.M., M.S.
National Toxicology Program
M.M. Gruebbel, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
D.E. Malarkey, D.V.M., Ph.D.
National Toxicology Program
T. Osborne, D.V.M., Ph.D.
National Toxicology Program
M.V. Pino, D.V.M., Ph.D.
Sanofi-aventis
D.B. Rao, BVSc, M.S., Ph.D.
ILS, Inc.
L.M. Staska, D.V.M., Ph.D.
ILS, Inc.

Evaluated slides and contributed to pathology report on 2-year mice (May 6, 2014)

L.M. Staska, D.V.M., Ph.D., Coordinator
ILS, Inc.
M.F. Cesta, D.V.M., Ph.D.
National Toxicology Program
T. Crabbs, D.V.M.
Experimental Pathology Laboratories, Inc.
S.A. Elmore, D.V.M., M.S.
National Toxicology Program
G.P. Flake, M.D.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
G.D. Hill, D.V.M., Ph.D.
ILS, Inc.
K.S. Janardhan, BVSc, MVSc, Ph.D.
ILS, Inc.
D.B. Rao, BVSc, M.S., Ph.D.
ILS, Inc.
R.A. Renne, D.V.M.
ToxPath Consulting

Social & Scientific Systems, Inc.
Provided statistical analyses

M.V. Smith, Ph.D., Principal Investigator
L.J. Betz, M.S.
S.F. Harris, B.S.

Biotechnical Services, Inc.
Prepared Technical Report

S.R. Gunnels, M.A., Principal Investigator
L.M. Harper, B.S.
P. Nader, B.S.E.
D.C. Serbus, Ph.D.

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SUMMARY

Background

TRIM VX is a metalworking fluid used as a lubricant and coolant liquid for cleaning tools and parts during cutting, drilling, milling, and grinding. Occupational exposures to metalworking fluids, including TRIM VX, occur primarily by dermal contact and inhalation. Inhalation exposure to other metalworking fluids has been reported to cause respiratory problems and to be associated with other health effects. We conducted studies of TRIM VX in rats and mice to determine if it caused cancer or other health effects.

Methods

We exposed groups of 50 male and female rats and mice to atmospheres containing aerosols of 10, 30, or 100 mg of TRIM VX per cubic meter of air. Similar groups of animals exposed to clean air in the same type of inhalation chambers served as the control groups. Animals were exposed six hours per day, five days per week for two years. Tissues from more than 40 sites were examined for every animal.

Results

The respiratory tract was the primary site of toxicity in all groups of male and female rats and mice exposed to TRIM VX. A wide spectrum of lesions (i.e., hyperplasia, inflammation, metaplasia, fibrosis) of the lung, nose, larynx, and lymph nodes was seen. The lesions were significantly increased across exposed groups of rats and mice, and many were observed at the lowest concentration tested (10 mg/m^3). In addition to these lesions, there were low occurrences of lung tumors in male and female rats and increases in the incidences of lung tumors in male and female mice exposed to the highest concentration of TRIM VX.

Conclusions

We conclude that exposure to aerosols of TRIM VX caused tumors of the lung in male and female mice and may have caused tumors of the lung in male and female rats. TRIM VX also caused a spectrum of lesions in the respiratory tract of male and female rats and mice.

ABSTRACT

TRIM® VX

TRIM VX is a metalworking fluid used as a lubricant and coolant liquid and for cleaning tools and parts during cutting, drilling, milling, and grinding. The metalworking fluid class was nominated by the National Institute for Occupational Safety and Health (NIOSH) for study by the National Toxicology Program because of high production volumes, the large number of occupationally exposed workers, the lack of carcinogenicity and chronic toxicology data, and because epidemiologic data indicate an increased incidence of laryngeal cancer in workers exposed to metalworking fluids. TRIM VX was selected as an example soluble oil metalworking fluid following chemical analysis and collaboration with NIOSH. Male and female Wistar Han [Crl:WI (Han)] rats and B6C3F1/N mice were exposed to TRIM VX by inhalation for 3 months or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, and rat and mouse peripheral blood erythrocytes.

3-Month Study in Rats

Groups of 10 male and 10 female rats were exposed by whole body inhalation to TRIM VX aerosol at concentrations of 0, 25, 50, 100, 200, or 400 mg/m^3 for 6 hours plus T_{90} (10 minutes) per day, 5 days per week for 14 weeks. All rats survived to the end of the study. The final mean body weight was significantly less in the 25 mg/m^3 males. In addition, the final mean body weight and the mean body weight gain of 400 mg/m^3 males were significantly less than those of the chamber controls. The absolute and relative lung weights of females exposed to 100 mg/m^3 or greater were significantly greater than those of the chamber controls. The absolute and relative liver weights of 200 and 400 mg/m^3 females and the relative liver weights of 200 and 400 mg/m^3 males were significantly increased; however, no histopathologic changes were noted.

In the lung, there were significantly increased incidences of fibrosis and chronic active inflammation in males and females exposed to 50 mg/m^3 or greater and histiocytic cellular infiltration in males and females exposed to 100 mg/m^3 or greater. In the nose, there were significantly increased incidences of suppurative inflammation, olfactory epithelium hyaline droplet accumulation, and respiratory epithelium hyaline droplet accumulation in all exposed groups of males and females compared to those in the chamber control groups. There were also significantly increased incidences of goblet cell hyperplasia, and hyperplasia and squamous metaplasia of the respiratory epithelium in 200 and 400 mg/m^3 males and females. In the larynx, there were significantly increased incidences of squamous hyperplasia, squamous metaplasia, and chronic active inflammation in all exposed groups of males and females.

3-Month Study in Mice

Groups of 10 male and 10 female mice were exposed by whole body inhalation to TRIM VX aerosol at concentrations of 0, 25, 50, 100, 200, or 400 mg/m^3 for 6 hours plus T_{90} (10 minutes) per day, 5 days per week for 14 weeks. All mice survived to the end of the study. The mean body weights of 400 mg/m^3 males were significantly less than those of the chamber controls. The absolute and relative lung weights of 200 and 400 mg/m^3 males and of 100 mg/m^3 or greater females were significantly increased. The absolute liver weights of 200 and 400 mg/m^3 males and females were significantly greater than those of the chamber control groups; the relative liver weights of all exposed male groups and of 200 and 400 mg/m^3 females were also significantly increased. The absolute and relative spleen weights of males exposed to 50 mg/m^3 or greater and the relative spleen weights of 400 mg/m^3 females were significantly increased. There was no evidence of histologic changes in the liver or spleen.

In the lung, the incidences of fibrosis and histiocytic cellular infiltration in males and females exposed to 100 mg/m^3 or greater were significantly greater than those in the chamber control groups. There were significantly increased incidences of chronic active inflammation in males exposed to 50 mg/m^3 or greater and females exposed to 100 mg/m^3 or greater and bronchiole hyperplasia in males and females exposed to 50 mg/m^3 or greater. In the nose, there were significantly increased incidences of hyaline droplet accumulation of the olfactory and respiratory epithelium in all exposed groups of males and females and suppurative inflammation in all

exposed groups of males and in females exposed to 50 mg/m^3 or greater. In the larynx, there were significantly increased incidences of squamous metaplasia in all exposed groups of males and females and squamous hyperplasia in males and females exposed to 100 mg/m^3 or greater. There were significantly increased incidences of chronic inflammation in all exposed groups of females.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female rats were exposed by whole body inhalation to TRIM VX aerosol at concentrations of 0, 10, 30, or 100 mg/m^3 for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks. Survival and mean body weights of all exposed groups were similar to those of the chamber control groups.

In the 100 mg/m^3 groups, alveolar/bronchiolar carcinomas occurred in two males and alveolar/bronchiolar adenomas occurred in one male and three females. There were significantly increased incidences of alveolar epithelium hyperplasia, alveolar/bronchiolar epithelium hyperplasia, fibrosis, histiocytic cellular infiltration, and chronic active inflammation in all exposed groups of males and females. There were significantly increased incidences of alveolar epithelium squamous metaplasia and lymphohistiocytic hyperplasia of bronchus-associated lymphoid tissue in 100 mg/m^3 males and 30 and 100 mg/m^3 females and alveolus proteinosis in 30 and 100 mg/m^3 males and all exposed groups of females.

In the nose, there were significantly increased incidences of olfactory epithelium glands hyperplasia, goblet cell hyperplasia, suppurative inflammation, and olfactory and respiratory epithelium hyaline droplet accumulation in all exposed groups of males and females. There were also significantly increased incidences of respiratory epithelium hyperplasia in 100 mg/m^3 males and all exposed groups of females and transitional epithelium hyperplasia in 100 mg/m^3 females.

In the larynx, there were significantly increased incidences of epiglottis squamous hyperplasia, epiglottis squamous metaplasia, and mixed cell infiltration in all exposed male and female groups.

There were significantly increased incidences of lymphohistiocytic hyperplasia in the bronchial and mediastinal lymph nodes in all exposed groups of males and females.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female mice were exposed by whole body inhalation to TRIM VX aerosol at concentrations of 0, 10, 30, or 100 mg/m^3 for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks. Survival and mean body weights of all exposed groups were similar to those of the chamber control groups.

The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in 100 mg/m^3 males and females and of alveolar/bronchiolar carcinoma in 100 mg/m^3 females were significantly increased compared to the chamber control incidences. There were significantly increased incidences of alveolar/bronchiolar epithelium hyperplasia, histiocytic cellular infiltration, and chronic inflammation in 30 and 100 mg/m^3 males and females, alveolar epithelium hyperplasia in 100 mg/m^3 males and females, and fibrosis in 30 and 100 mg/m^3 males and 100 mg/m^3 females.

In the nose, there were significantly increased incidences of exudate, chronic active inflammation, and olfactory epithelium hyaline droplet accumulation in all exposed male and female groups. In the respiratory epithelium, there were significantly increased incidences of hyaline droplet accumulation in all exposed groups of males and females, atrophy in 30 and 100 mg/m^3 males and females, and necrosis in 100 mg/m^3 males and 30 and 100 mg/m^3 females. The incidences of turbinate atrophy in 30 and 100 mg/m^3 males and females, turbinate perforation in 100 mg/m^3 males and 30 and 100 mg/m^3 females, and nasopharyngeal duct perforation in 30 and 100 mg/m^3 males and females were also significantly increased.

In the larynx, there were significantly increased incidences of squamous hyperplasia of the epiglottis in 30 and 100 mg/m^3 males and females and squamous metaplasia of the epiglottis in all exposed groups of males and females.

In the bronchial lymph node of 100 mg/m^3 males, there were significantly increased incidences of lymphoid hyperplasia and histiocytic cellular infiltration.

GENETIC TOXICOLOGY

TRIM VX gave no evidence of genotoxicity in bacterial mutation tests or *in vivo* tests for chromosomal damage (micronuclei). No mutagenic activity was observed with

TRIM VX in *S. typhimurium* strains TA98 or TA100 or in *E. coli* strain WP2 *uvrA*/pKM101, with or without exogenous metabolic activation (induced rat liver S9). In tests for induction of chromosomal damage *in vivo*, no increases in the frequencies of micronucleated reticulocytes or erythrocytes were seen in peripheral blood samples from male or female rats or mice exposed to TRIM VX by inhalation for 3 months.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *equivocal evidence of carcinogenic activity** of TRIM VX in male Wistar Han rats based on the combined occurrences of alveolar/bronchiolar adenoma or carcinoma of the lung. There was *equivocal evidence of carcinogenic activity* of TRIM VX in female Wistar Han rats based on the occurrences of alveolar/bronchiolar adenoma of the lung. There was *clear evidence of carcinogenic activity* of TRIM VX in male B6C3F1/N mice based on the increased combined incidences of alveolar/bronchiolar adenoma or carcinoma of the lung. There was *clear evidence of carcinogenic activity* of TRIM VX in female B6C3F1/N mice based on the increased combined incidences of alveolar/bronchiolar adenoma or carcinoma (primarily carcinoma) of the lung.

Exposure to TRIM VX resulted in increased incidences of nonneoplastic lesions of the lung, nose, and larynx in male and female rats and mice, the bronchial lymph node in male and female rats and male mice, and the mediastinal lymph node in male and female rats.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Peer Review Panel comments and the public discussion on this Technical Report appears in Appendix K.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of TRIM VX

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations in air	0, 10, 30, or 100 mg/m^3	0, 10, 30, or 100 mg/m^3	0, 10, 30, or 100 mg/m^3	0, 10, 30, or 100 mg/m^3
Survival rates	36/50, 39/50, 33/50, 34/50	30/50, 33/50, 33/50, 30/50	38/50, 39/50, 37/50, 37/50	35/50, 36/50, 36/50, 30/50
Body weights	Exposed groups similar to the chamber control group	Exposed groups similar to the chamber control group	Exposed groups similar to the chamber control group	Exposed groups similar to the chamber control group
Nonneoplastic effects	Lung: alveolar epithelium, hyperplasia (11/50, 43/50, 45/50, 49/50); alveolar/ bronchiolar epithelium, hyperplasia (4/50, 22/50, 39/50, 46/50); fibrosis (4/50, 43/50, 45/50, 49/50); infiltration cellular, histiocyte (14/50, 50/50, 50/50, 50/50); inflammation, chronic active (7/50, 46/50, 46/50, 48/50); alveolar epithelium, metaplasia, squamous (0/50, 0/50, 0/50, 5/50); bronchus-associated lymphoid tissue, hyperplasia, lymphohistiocytic (0/50, 1/50, 4/50, 6/50); alveolus, proteinosis (0/50, 1/50, 31/50, 45/50) Nose: glands, olfactory epithelium, hyperplasia (0/50, 43/50, 41/50, 49/50); goblet cell, hyperplasia (5/50, 36/50, 37/50, 42/50); inflammation, suppurative (7/50, 46/50, 47/50, 46/50); olfactory epithelium, accumulation, hyaline droplet (20/50, 50/50, 50/50, 50/50); respiratory epithelium, accumulation, hyaline droplet (10/50, 50/50, 48/50, 50/50); respiratory epithelium, hyperplasia (2/50, 7/50, 7/50, 19/50)	Lung: alveolar epithelium, hyperplasia (8/50, 43/50, 49/50, 50/50); alveolar/ bronchiolar epithelium, hyperplasia (2/50, 9/50, 31/50, 50/50); fibrosis (5/50, 35/50, 49/50, 50/50); infiltration cellular, histiocyte (16/50, 48/50, 50/50, 50/50); inflammation, chronic active (5/50, 46/50, 50/50, 50/50); alveolar epithelium, metaplasia, squamous (0/50, 3/50, 9/50, 21/50); bronchus-associated lymphoid tissue, hyperplasia, lymphohistiocytic (0/50, 2/50, 7/50, 10/50); alveolus, proteinosis (1/50, 15/50, 41/50, 48/50) Nose: glands, olfactory epithelium, hyperplasia (0/50, 32/50, 43/49, 46/50); goblet cell, hyperplasia (3/50, 36/50, 40/49, 47/50); inflammation, suppurative (1/50, 46/50, 47/49, 48/50); olfactory epithelium, accumulation, hyaline droplet (14/50, 50/50, 49/49, 50/50); respiratory epithelium, accumulation, hyaline droplet (5/50, 50/50, 48/49, 50/50); respiratory epithelium, hyperplasia (0/50, 7/50, 8/49, 25/50); transitional epithelium, hyperplasia (5/50, 11/50, 4/49, 21/50)	Lung: alveolar/bronchiolar epithelium, hyperplasia (3/50, 7/50, 15/49, 50/50); infiltration cellular, histiocyte (5/50, 9/50, 15/49, 49/50); inflammation, chronic (5/50, 12/50, 16/49, 50/50); alveolar epithelium, hyperplasia (3/50, 3/50, 7/49, 47/50); fibrosis (0/50, 2/50, 5/49, 45/50) Nose: exudate (2/49, 11/50, 35/49, 49/50); inflammation, chronic active (3/49, 33/50, 39/49, 50/50); olfactory epithelium, accumulation, hyaline droplet (2/49, 46/50, 48/49, 50/50); respiratory epithelium, accumulation, hyaline droplet (7/49, 49/50, 49/49, 50/50); respiratory epithelium, atrophy (0/49, 1/50, 20/49, 40/50); respiratory epithelium, necrosis (2/49, 1/50, 2/49, 23/50); turbinate, atrophy (0/49, 2/50, 5/49, 14/50); turbinate, perforation (0/49, 0/50, 1/49, 13/50); nasopharyngeal duct, perforation (0/49, 1/50, 11/49, 19/50) Larynx: epiglottis, hyperplasia, squamous (1/48, 2/49, 14/49, 30/49); epiglottis, metaplasia, squamous (0/48, 49/49, 49/49, 49/49)	Lung: alveolar/bronchiolar epithelium, hyperplasia (0/50, 3/50, 8/50, 45/50); infiltration cellular, histiocyte (1/50, 4/50, 15/50, 48/50); inflammation, chronic (1/50, 6/50, 26/50, 47/50); alveolar epithelium, hyperplasia (0/50, 0/50, 2/50, 43/50); fibrosis (0/50, 0/50, 2/50, 42/50) Nose: exudate (8/50, 17/50, 48/50, 49/50); inflammation, chronic active (4/50, 25/50, 49/50, 49/50); olfactory epithelium, accumulation, hyaline droplet (14/50, 48/50, 50/50, 50/50); respiratory epithelium, accumulation, hyaline droplet (23/50, 50/50, 50/50, 50/50); respiratory epithelium, atrophy (1/50, 2/50, 28/50, 39/50); respiratory epithelium, necrosis (0/50, 2/50, 13/50, 23/50); turbinate, atrophy (0/50, 0/50, 10/50, 19/50); turbinate, perforation (0/50, 0/50, 6/50, 6/50); nasopharyngeal duct, perforation (0/50, 0/50, 14/50, 17/50) Larynx: epiglottis, hyperplasia, squamous (4/50, 3/50, 16/50, 42/50); epiglottis, metaplasia, squamous (0/50, 50/50, 50/50, 50/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of TRIM VX

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Nonneoplastic effects (continued)	Larynx: epiglottis, hyperplasia, squamous (0/50, 26/50, 48/50, 50/50); epiglottis, metaplasia, squamous (3/50, 50/50, 50/50, 50/50); infiltration cellular, mixed cell (1/50, 9/50, 27/50, 31/50) Lymph node, bronchial: hyperplasia, lymphohistiocytic (0/39, 17/43, 29/42, 35/42) Lymph node, mediastinal: hyperplasia, lymphohistiocytic (0/43, 20/48, 22/44, 32/43)	Larynx: epiglottis, hyperplasia, squamous (1/50, 24/50, 41/50, 50/50); epiglottis, metaplasia, squamous (0/50, 49/50, 50/50, 50/50); infiltration cellular, mixed cell (0/50, 9/50, 18/50, 22/50) Lymph node, bronchial: hyperplasia, lymphohistiocytic (1/37, 18/37, 37/44, 31/36) Lymph node, mediastinal: hyperplasia, lymphohistiocytic (0/46, 11/49, 14/46, 28/45)	Lymph node, bronchial: hyperplasia, lymphoid (3/38, 3/37, 2/39, 14/39); infiltration cellular, histiocyte (0/38, 1/37, 0/39, 7/39)	
Neoplastic effects	None	None	Lung: alveolar/bronchiolar adenoma or carcinoma (14/50, 14/50, 11/49, 23/50)	Lung: alveolar/bronchiolar carcinoma (5/50, 3/50, 6/50, 14/50); alveolar/bronchiolar adenoma or carcinoma (9/50, 8/50, 8/50, 20/50)
Equivocal findings	Lung: alveolar/bronchiolar carcinoma (0/50, 0/50, 0/50, 2/50); alveolar/bronchiolar adenoma or carcinoma (0/50, 0/50, 0/50, 3/50)	Lung: alveolar/bronchiolar adenoma (0/50, 0/50, 1/50, 3/50)	None	None
Level of evidence of carcinogenic activity	Equivocal evidence	Equivocal evidence	Clear evidence	Clear evidence

Genetic toxicology

Bacterial gene mutations:	Negative in *S. typhimurium* strains TA98 and TA100 and in *E. coli* strain WP2 *uvrA*/pKM101 with and without S9
Micronucleated erythrocytes	
Rat peripheral blood *in vivo*:	Negative in males and females
Mouse peripheral blood *in vivo*:	Negative in males and females

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that cannot be evaluated because of major flaws **(inadequate study)**. These categories of interpretative conclusions were first adopted in June 1983 and then revised on March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft NTP Technical Report on TRIM VX on February 16, 2016, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. A summary of the minutes from the Peer Review can be found in Appendix K. In this capacity, panel members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

Jon C. Mirsalis, Ph.D., Chairperson
SRI International
Menlo Park, CA

William J. Brock, Ph.D., Primary Reviewer
Brock Scientific Consulting, LLC
Montgomery Village, MD

Michael R. Elwell, D.V.M., Ph.D., Primary Reviewer
Nonclinical Safety Assessment
Covance Laboratories, Inc.
Chantilly, VA

Kent E. Pinkerton, Ph.D.
Center for Health and the Environment
University of California
Davis, CA

Michael V. Pino, D.V.M., Ph.D.
Veterinary Toxicologic Pathology and Preclinical Drug Development
Albuquerque, NM

Tara Sabo-Attwood, Ph.D., Primary Reviewer
Department of Environmental and Global Health
University of Florida
Gainesville, FL

Madhuri Singal, Ph.D.
Global Regulatory Compliance
Reckitt Benckiser
Montvale, NJ

OVERVIEW

The National Institute for Occupational Safety and Health (NIOSH) nominated the class of metalworking fluids to the National Toxicology Program (NTP) for toxicological evaluation. Individual metalworking fluids are formulated based upon the intended use, and there are hundreds, and perhaps many more, metalworking fluid formulations currently in use. Selection of specific metalworking fluids for study based upon production volumes and use patterns was not possible because this information is proprietary thus was not available. For this reason, selection of specific metalworking fluid formulations was accomplished by initially selecting 30 prominently marketed metalworking fluids from among the three major classes of metalworking fluids: soluble oils, semisynthetic fluids, and synthetic fluids. These three classes encompass the majority of metalworking fluids in use. From the initial 30 metalworking fluids, 18 were selected for chemical analysis based on commercial availability, review of composition as listed on Material Safety Data Sheets, and elimination of redundant formulations. The number of formulations for study was reduced to nine by selecting from a minimum of three major producers following chemical screening. These nine formulations underwent further chemical analysis and genetic toxicity assessment prior to selecting four formulations, as examples from each class, for inhalation toxicity studies. In collaboration with NIOSH, the selection for the NTP studies was based on class, estimated usage, and chemical composition. CIMSTAR® 3800 (semisynthetic), TRIM® VX (soluble oil), Syntilo® 1023 (synthetic), and TRIM® SC210 (semisynthetic) were selected for 3-month inhalation studies. Based on the results of the 3-month studies, CIMSTAR® 3800 and TRIM® VX were selected for 2-year studies (Ryan *et al*., 2016). CIMSTAR® 3800 3-month and 2-year studies are reported in TR 586 (NTP, 2015). TRIM® VX 3-month and 2-year studies are reported in the current Technical Report (TR 591). Although registered trademarks, the formulations may be be referred to as TRIM, CIMSTAR, and Syntilo throughout this document.

INTRODUCTION

TRIM® VX

Chemical and Physical Properties

TRIM VX, like many other metalworking fluids, is a highly complex mixture containing oils, detergents, surfactants, biocides, lubricants, anticorrosive agents, and other ingredients. Metalworking fluids are designed to enhance machining processes such as lubricating and cooling or for cleaning tools and parts during cutting, drilling, milling, and grinding. Therefore, chemical species present in these mixtures are dependent on requirements of the particular machining process. Metalworking fluids are classified into the general categories of straight oils, soluble oils, semisynthetic fluids, or synthetic fluids based upon the amount of highly refined oil they contain (Meyer and Boslett, 2001; Gauthier, 2003; Woskie *et al*., 2003). Straight oils contain 60% to 100% oil derived from highly refined napthenic or paraffinic oils. The soluble (emulsifiable) oils and semisynthetic fluids contain 5% to 85% oil, while the synthetic fluids contain no oil and are 70% to 95% water (Cohen and White, 2006). TRIM VX is classified as a soluble oil metalworking fluid because it is primarily oil but does contain approximately 7% water. Generally, the concentrated soluble metalworking fluids are diluted prior to use with water at ratios of one part concentrate to five to 40 parts water. These solutions form oil-water emulsions with the water acting as a cooling agent and the oil providing lubrication for enhanced heavy duty machining operations.

The specific chemical composition of TRIM VX and other metalworking fluids is considered proprietary information by the metalworking fluid industry. However, some typical components of soluble oil metalworking fluids include mineral oils, fatty acids, sulfur, chlorine, and phosphorus derivatives to provide enhanced lubrication (Frazier, 1982; Pryce *et al*., 1989). Other additives can include detergents to lower surface tension, polyol ester as an "oiliness agent," petroleum sulfonate as an emulsifier, alkanolamines to provide reserve alkalinity and inhibit corrosion, chlorinated paraffins as "extreme pressure agents," long chain fatty alcohols as defoamers, triazoles as metal passivators, antioxidants, dyes, and biocides (Gordon, 2004).

Metalworking fluids, including TRIM VX, are commonly recycled or reused because they are expensive and single use is not practical. Long-term physical and chemical effects can cause the components of metalworking fluids to deteriorate, and as a result, the composition of these "in-use" metalworking fluids is considerably different from unused metalworking fluids. For example, excessive temperatures can cause oxidation of metalworking fluid oils and constituents leading to the formation of acids, resins, varnishes, sludge, and carbonaceous deposits. Furthermore, depletion of performance additives with use requires routine product addition or supplementation. Finally, environmental contaminants including rust, weld spatter, metal chips and abrasives, as well as contaminants entering through broken seals, dirty oil filter pipes, and chemical residue on metal parts can accelerate metalworking fluid breakdown.

Contamination of in-use metalworking fluids with bacteria, bacterial endotoxins, and fungi is an added concern for individuals working with these chemicals. Contaminated metalworking fluids and aerosols increase the potential for respiratory effects when inhaled and skin infections following dermal contact. Antimicrobial agents are often added to metalworking fluids to control the growth of microbes and to extend the usable life of these fluids. However, the microbiocidal or microbiostatic activities of some antimicrobial agents occur through the release of formaldehyde, which is an airway irritant and a recognized cause of occupational asthma (Arrandale *et al*., 2012). Studies suggest that exposure to certain antimicrobial agents can cause allergic or irritant contact dermatitis (Zugerman, 1986; Pryce *et al*., 1989). TRIM VX reportedly contains the biocide 4-chloro-3-methylphenol (CMP), which was identified as a potent sensitizer in a combined local lymph node assay (Anderson *et al*., 2009), and is reported to be irritating to the eyes, skin, and respiratory tract following short-term exposure (CDC, 2014).

Formulations of metalworking fluids are continuously changing to improve functionality and reduce potential health and environmental concerns. Examples of chemicals reduced or removed over time include rust inhibitors (nitrites), biocides, corrosion inhibitors [diethanolamines (DEA), 4-tert-butylbenzoic acid, dichromates], and lubricants such as polychlorinated biphenyls (Cohen and White, 2006). Efforts have been taken to remove components with suspected carcinogenicity such as polycyclic aromatic hydrocarbons (PAHs), nitrosating agents and short-chained chlorinated paraffin from metalworking fluids from 1950 to 1990 by the United States Environmental Protection Agency (40 CFR, § 747.115). In addition, the concentrations of some suspected carcinogens, such as ethanolamine, have been reduced in some metalworking fluids as an attempt to reduce exposure-related health hazards. Efforts to reduce volatile organic compounds have resulted in the increased use of water as a major component of currently used metalworking fluids. Toxicity and carcinogenicity data on older metalworking fluids may not apply to metalworking fluids currently in use. Although many carcinogenic components have been removed, the newer metalworking fluids have not been tested so the current cancer risk is unclear.

PRODUCTION, USE, AND HUMAN EXPOSURE

The production of metalworking fluids started in the early 1900s (Newhouse, 1982). Today, specific data available on metalworking fluid (e.g., TRIM VX) production and use are not available as this information is considered proprietary by the metalworking fluid manufacturers. However, the Independent Lubricant Manufacturers Association (ILMA, 2000) reported that 95 to 103 million gallons of metalworking fluids were produced annually in the United States from 1994 to 1999. In 1999, five companies each reported producing more than 5 million gallons of these fluids. The largest use of metalworking fluids in terms of industry consumption is the metal removal fluids with more than 100 million gallons consumed annually (ILMA, 2000).

Metalworking fluids are primarily used to reduce friction, minimize heat generation, and extend tool life in metalworking operations. Most soluble oil metalworking fluids, such as TRIM VX, are recommended for use in heavy duty machining operations to prevent welding of the cutting tool to the work surface, reduce abrasive wear of the tool at high temperatures, and prevent distortion caused by residual heat. The manufacturer recommends diluting TRIM VX concentrate 5% to 20% in water before use (Master Chemical Corporation, 2014).

The National Occupational Exposure Survey (NIOSH, 1990) estimated that over 1.2 million workers were potentially exposed to metalworking fluids from 1981 to 1983. Health hazard evaluations (HHEs) from the National Institute for Occupational Safety and Health (NIOSH, 2013a) indicate that exposures to metalworking fluid aerosols have decreased over time but it is unclear how many current workers are potentially exposed. HHEs conducted by NIOSH from approximately 1970 to 1990 indicate an overall exposure mean concentration of 0.96 mg/m^3 across 38 individual plants using metalworking fluids (NIOSH, 2001). Industry-sponsored exposure reports from three investigations indicated an arithmetic mean metalworking fluid airborne exposure concentration of less than 1.0 mg/m^3 (Kriebel *et al.*, 1994; Robins *et al.*, 1994; Greaves *et al.*, 1997).

Exposure occurs primarily from inhalation of aerosols and dermal contact with aerosols or when handling equipment covered in metalworking fluids. Workers can receive significant exposure to metalworking fluids by inhalation of aerosols (Bennett and Bennett, 1987). Aerosols form when a metalworking fluid is subjected to high shear forces or excess heat during use. The characteristics of these aerosols are dependent upon the particular metalworking fluid and the machining process for which it is being used; however, some aerosols can be stable and long lasting (Simpson *et al.*, 2003). Dermal exposure to metalworking fluids can occur through contact with contaminated equipment, from splashes, or exposure to aerosols. The components and alkalinity of metalworking fluids are well-known causes of dermatitis in workers (Geier *et al.*, 2004; Suuronen *et al.*, 2007). There are no specific estimates of TRIM VX human exposure reported in the literature.

REGULATORY STATUS

NIOSH recommends an exposure limit (REL) for all metalworking fluid aerosols of 0.4 mg/m^3 for thoracic particulate mass. The NIOSH REL for total particulate mass is 0.5 mg/m^3 as a time-weighted average concentration up to 10 hours per day during a 40-hour workweek (NIOSH, 1998). Metalworking fluid mists are regulated as a nuisance dust by the Occupational Safety and Health Administration (OSHA) and the permissible exposure limit is 15 mg/m^3 for total particulate (OSHA, 2001). There are no specific regulations for TRIM VX use and exposure itself. The Material Safety Data Sheet provided by the manufacturer states that all TRIM VX ingredients are listed on the Toxic Substances Control Act Inventory of Chemical Substances.

Absorption, Distribution, Metabolism, and Excretion

No studies evaluating the absorption, distribution, metabolism, or excretion (ADME) of metalworking fluids including TRIM VX in experimental animals or humans were found in the literature. For some of the components of TRIM VX, there are ADME data available in the literature; however, it is not the intent to discuss each individual component here.

Toxicity

Experimental Animals

Respiratory toxicity attributed to TRIM VX exposure was not found in the literature; however, several animal models have been used to evaluate different classes of metalworking fluids. Schaper and Detwiler (1991) used Swiss Webster mice to compare the effects of different classes of metalworking fluids on pulmonary function. Mice were exposed for 3 hours at concentrations ranging from 20 to 200 mg/m^3. Results from this study demonstrated that all 10 proprietary metalworking fluids examined were sensory and pulmonary irritants. Increased breathing rate was most significant after inhalation of semisynthetic and synthetic fluids followed by soluble oils and finally straight oil aerosols. Furthermore, pulmonary irritation was similar in mice exposed to unused and used metalworking fluids. In a subsequent study (Schaper and Detwiler-Okabayashi, 1995), the same mouse model was used to evaluate the sensory and pulmonary irritation properties of a single metalworking fluid from the 1991 study (classified as "neat, soluble oil") and to identify the contribution of three major components. Results indicated that sensory irritation was caused mainly by tall oil fatty acids, whereas pulmonary irritation was caused primarily by sodium sulfonate (i.e., corrosion inhibitor). Paraffinic oils in the metalworking fluid decreased the irritant properties of both tall oil fatty acids and sodium sulfonate.

The subchronic respiratory toxicity of metalworking fluids has been investigated in several studies. Lim *et al.* (2005a) exposed F344 rats to a water-soluble metalworking fluid at aerosol concentrations of 0, 20, 60, or 180 mg/m^3 6 hours/day, 5 days per week for 13 weeks. At 60 and 180 mg/m^3, pulmonary inflammation consisting of increases in lung weights, bronchoalveolar lavage (BAL) fluid neutrophils, foamy macrophages, and thickening of the alveolar walls was observed in the absence of clinical observations. In a more recent subchronic study, male and female F344/NTac rats and B6C3F1/N mice were exposed to aerosol concentrations of 25 to 400 mg/m^3 CIMSTAR 3800, a semisynthetic metalworking fluid, for 13 weeks (NTP, 2015). Results of this study demonstrated toxicity to the respiratory tract of both rats and mice but no effect on survival. In the nasal cavity of rats, there were significantly increased incidences of goblet cell hyperplasia and hyaline droplet accumulation and suppurative inflammation of the olfactory and respiratory epithelia in all exposed groups of males and females. Mice had fewer lesions in the nose which included hyaline droplet accumulation of the olfactory and respiratory epithelia in all exposed groups. In the larynx of rat and mice, there were significantly increased incidences of squamous metaplasia, hyperplasia of the squamous epithelium, and chronic active inflammation in all exposed groups of rats and some exposed groups of mice. The incidences of epiglottis dysplasia were also significantly increased in the larynx of male and female mice exposed to 400 mg/m^3 CIMSTAR 3800. In the lung, rats had fewer lesions than mice. In rats, there were significantly increased incidences of alveolus histiocytic cellular infiltration in male and females exposed to 200 or 400 mg/m^3, while mice had significantly increased incidences of bronchiole hyperplasia in all exposed groups and perivascular chronic active inflammation and arteriole hypertrophy in some of the groups exposed to 100 mg/m^3 or greater.

The acute and subacute effects of in-use metalworking fluids contaminated with endotoxins have also been investigated in experimental animals by several groups. Thorne and DeKoster (1996) reported that in-use metalworking fluids were consistently more toxic than neat metalworking fluids. In this report, acute inhalation exposure of mice to a high concentration of in-use metalworking fluid aerosol contaminated with endotoxin caused a significant elevation of neutrophils, TNF-α, and IL-6 in BAL fluid. DeLorme *et al.* (2003) demonstrated that acute exposure to water-soluble metalworking fluids containing endotoxin also caused an increase in BAL fluid neutrophils in Wistar rats. Sprague Dawley rats were exposed to water-soluble metalworking fluids with and without endotoxin for 2 weeks (Lim *et al.*, 2005b). In this study, endotoxin-contaminated metalworking fluids caused a significantly elevated inflammatory response in the lung relative to uncontaminated metalworking fluid. Furthermore, the same investigators reported that exposure of F344 rats to endotoxin-contaminated metalworking fluids for 8 weeks increased lung inflammatory responses in the absence of altered pulmonary function (Lim *et al.*, 2005c).

Dermal sensitization and irritancy potential of nine metalworking fluids, including TRIM VX, were investigated in a combined local lymph node assay using female BALB/c mice (Anderson *et al.*, 2009). Significant irritation was observed after dermal exposure to seven of the nine metalworking fluids. TRIM VX was identified as an irritant at concentrations as low as 10%. In addition, six of the nine metalworking fluids tested induced a greater than threefold increase in lymphocyte proliferation; TRIM VX induced the most robust proliferative response. Several components of TRIM VX were identified by high performance liquid chromatography and were screened for sensitization potential using structure activity relationship (SAR) modeling and the local lymph node assay. SAR modeling predicted two TRIM VX components, triethanolamine (TEA) and 4-chloro-3-methylphenol (CMP), to be positive for sensitization. CMP and oleic acid stimulated lymphocyte proliferation but only at doses that resulted in significant irritancy.

Individual components of metalworking fluids and their potential to cause toxicity have been investigated in various experimental animal models. While it is not in the scope of this report to discuss the results, some of the data are summarized by NIOSH (2001).

Humans

Occupational exposure to metalworking fluid aerosols is associated with a variety of nonmalignant respiratory and dermal conditions, however, there are no specific data linking TRIM VX exposure with toxicity in humans. Occupational exposure to metalworking fluid aerosols causes symptoms consistent with airway irritation, chronic bronchitis, and asthma. The epidemiologic evidence suggests that at least three classes of metalworking fluids (straight oil, soluble oil, and synthetic) are capable of inducing respiratory symptoms. Hypersensitivity pneumonitis, asthma, and allergic airways diseases are reported to be associated with metalworking fluid use and exposure in occupational settings (Mirer, 2010).

Hypersensitivity pneumonitis is characterized in its acute phase by alveolar inflammation and influenza-like symptoms. In its chronic phase (following repeated exposures), it is characterized by pulmonary fibrosis associated with respiratory insufficiency. Two cases of hypersensitivity pneumonitis associated with metalworking fluids were reported during a 3-year period in an occupational respiratory disease surveillance program operating in the United Kingdom (Meredith and McDonald, 1994). Many more cases in North America have been recognized (Rosenman *et al*., 1994; Bernstein *et al*., 1995; Rose *et al*., 1996; Kreiss and Cox-Ganser, 1997). Some causative agents for hypersensitivity pneumonitis are small organic particles or volatile reactive chemicals (Bogaert *et al*., 2009).

An elevated risk of asthma among workers exposed to metalworking fluid aerosols has been documented. Exposure to synthetic metalworking fluids has been associated with asthma (Eisen *et al*., 1997; Greaves *et al*., 1997; Rosenman *et al*., 1997) and case reports have documented asthma caused by exposure to soluble oil metalworking fluids (Hendy *et al*., 1985; Robertson *et al*., 1988) or to common components of soluble oil metalworking fluids (Savonius *et al*., 1994). For example, 13 case reports of occupational asthma attributed to soluble oil metalworking fluids were documented in a surveillance program in Michigan during 1988 to 1994 (Rosenman *et al*., 1997). A variety of components, additives, and contaminants of metalworking fluids are sensitizers or irritants known to induce new-onset asthma, aggravate preexisting asthma, or irritate the airways of non-asthmatic workers. These sensitizers, irritants, or toxicants include ethanolamine and other amines, colophony, pine oil, tall oil, metals and metallic salts (e.g., chromium, nickel, cobalt, and tungsten carbide), castor oil, formaldehyde, chlorine, various acids, and fungal and other microbial contaminants (including gram-negative bacterial endotoxin) (Hendy *et al*., 1985; Kennedy, 1992; Michel *et al*., 1992).

There is no convincing evidence that identifies any particular component or components of metalworking fluid aerosol as the predominant cause of respiratory symptoms, although some irritant components of metalworking fluid are clearly suspect (Sprince *et al*., 1997). One large multiplant study in the United States with mean exposures for the major types of metalworking fluids ranging from 0.41 to 0.55 mg/m^3 (thoracic fraction) found statistically significant quantitative exposure-response relationships between cumulative concentration of metalworking fluid aerosols and respiratory symptoms (Greaves *et al*., 1997). Likewise, another United States study found significant exposure-response relationships between aerosol exposure concentration and chest symptoms (Sprince *et al*., 1997). Other studies have shown the onset or worsening of many symptoms in workers over the course of a work shift (Kriebel *et al*., 1997; Rosenman *et al*., 1997; Sprince *et al*., 1997) and substantial symptomatic improvement in affected workers when away from work (Greaves *et al*., 1997). Controlling worker exposures may prevent chronic effects induced by metalworking fluid aerosol exposure and may reverse early metalworking fluid-induced airway effects.

In addition to respiratory conditions, occupational exposures to metalworking fluids are associated with irritant and allergic dermatitis in workers. The type and concentration of fluid and duration of use as well as the presence of preexisting skin disease may contribute to the development of dermatitis. The components and alkalinity of metalworking fluids are well-known causes of dermatitis in workers (Geier *et al*., 2004; Suuronen *et al*., 2007). The additives in metalworking fluids such as biocides, preservatives, corrosion inhibitors, amines and the impurities from metal (chrome, nickel), act as allergens and can cause an allergic reaction in some susceptible individuals.

Reproductive and Developmental Toxicity

No studies on metalworking fluid reproductive or developmental toxicity in experimental animals or humans were found in the literature. For some of the components of TRIM VX, there are reproductive and developmental toxicity studies available in the literature; however, it is not the intent to discuss each individual component here.

Carcinogenicity

Experimental Animals

There have been no carcinogenicity studies of TRIM VX in experimental animals. However several studies have examined the carcinogenicity of neat and used metalworking fluids in experimental animals (Gilman and Vesselinovitch, 1955; Jepsen *et al*., 1977; Wang *et al*., 1988; Gupta and Mehrotra, 1989; McKee *et al*., 1990; NTP, 2015). Gilman and Vesselinovitch (1955) applied soluble cutting oils formulated from unrefined distillates to the skin three times weekly for 310 days in three mouse strains and found that 19% to 61% of mice developed skin tumors while no tumors occurred in the skin of unexposed controls. Jepsen *et al*. (1977) compared various forms of paraffin- and naphthalene-based straight oil and solvent-extracted metalworking fluids in mice following dermal application for 31 weeks. The incidences of skin papillomas (40% to 100%) were more pronounced in mice treated with undiluted (i.e., straight oil) metalworking fluids versus diluted versions. This study also demonstrated differences in skin lesions following application of unused versus used metalworking fluids. In mice treated with unused solvent-extracted paraffin oil, 45% of the animals had papillomas whereas there was a 0% occurrence with the used form of the oil. However, the papilloma incidence was 80% to 100% in mice treated with naphthalene-based straight oil regardless of whether it was used or unused. Another study found that both unused and used cutting oils were potent skin tumor initiators (Gupta and Mehrotra, 1989). Among mice administered a single application of a cutting oil followed by a weekly application (three times a week) of the tumor promoting agent 12-*O*-tetradecanoylphorbol-13-acetate for 28 weeks, 90% and 60% of mice developed benign skin cancers after exposure to unused and used cutting oil, respectively. McKee *et al*. (1990) found no evidence of carcinogenicity from solvent-extracted cutting oils when they were administered to the skin of mice three times a week. The carcinogenic effects of metalworking fluid exposure following oral exposure were assessed by Wang *et al*. (1988). In the 2-year study in Wistar rats, pancreatic carcinoma occurred in nine of 40 rats dosed with undiluted rustproof cutting fluid consisting of sodium nitrite, TEA, and polyethylene glycol, while none of the control rats developed pancreatic cancer. All three of the components reported to be in this rustproof cutting fluid can be found in some metalworking fluids used in the United States. In a more recent study, groups of 50 male and 50 female Wistar Han rats and B6C3F1/N mice were exposed by whole body inhalation to aerosol concentrations of 0, 10, 30, or 100 mg/m^3 CIMSTAR 3800, a semisynthetic metalworking fluid, for 2 years (NTP, 2015). Survival and body weights of exposed groups of rats and mice were similar to those of the chamber control groups. Exposure to CIMSTAR 3800 resulted in increased incidences of nonneoplastic lesions of the nose, larynx, and lung in male and female rats and mice, lymph nodes in male and female rats, and thyroid gland in female mice. Increased incidences of prostate gland adenoma or carcinoma and of benign or malignant granular cell tumors of the brain occurred in exposed groups of male rats. Increased incidences of squamous cell papilloma or keratoacanthoma of the skin and of adenocarcinoma or mixed malignant Müllerian tumors of the uterus occurred in exposed groups of female rats. There was no evidence of carcinogenic activity of CIMSTAR 3800 in male mice; however, exposed groups of female mice had increased incidences of follicular cell carcinoma of the thyroid gland and of alveolar/bronchiolar adenoma or carcinoma of the lung.

Specific components of metalworking fluids such as DEA, TEA, nitrosoamines, and formaldehyde have been evaluated for carcinogenic potential on an individual basis. Variable amounts of alkanolamines are present in many metalworking fluids. The carcinogenicity of TEA was investigated because of its potential conversion to the carcinogen *N*-nitrosodiethanolamine. Following dermal exposure for 2 years, there was equivocal evidence of carcinogenic activity of TEA in male F344/N rats based on marginally increased incidences of renal tubule adenoma and no evidence of carcinogenicity in female rats (NTP, 1999a). In a 2-year dermal study of TEA in B6C3F1 mice, there was equivocal evidence of carcinogenicity in males based on the occurrence of liver hemangiosarcoma and some evidence of carcinogenicity in females based on increased incidences of hepatocellular adenoma (NTP, 2004). The carcinogenicity of DEA was also studied in F344/N rats and B6C3F1/N mice in 2-year dermal exposure studies (NTP, 1999b). There was no evidence of carcinogenic activity in rats; however, there was clear evidence of carcinogenic activity of DEA in mice based on increased incidences of liver neoplasms in males and females and increased incidences of renal tubule neoplasms in males.

Humans

No epidemiologic studies have evaluated the carcinogenicity of TRIM VX in humans. In most studies, metalworking fluids have been treated as a single exposure agent, without regard to type, constituents, or concentration. Evidence exists for increased risk of squamous cell skin cancer (Cruickshank and Gourevitch, 1952; Järvholm *et al*., 1985; Järvholm and Easton, 1990) especially of the scrotum (Cruickshank and Squire, 1950; Roush *et al*., 1982; Järvholm and Lavenius, 1987) following exposure to metalworking fluids. The International Agency for Research on Cancer (1987) designated unused and mildly used mineral oil as carcinogenic to humans based on squamous cell carcinoma of the skin, sinonasal cancer, and bladder cancer. NIOSH (1998) concluded that substantial evidence exists for an increased risk of cancer at several tissue sites (larynx, rectum, pancreas, skin, scrotum, and urinary bladder) associated with at least some of the metalworking fluids used before the mid-1970s. The inconsistencies between studies with respect to the tissue sites that were affected, and the variation in the strength of association between the surrogates of exposure and specific sites are most likely related to the diverse nature of the metalworking fluid mixtures studied, the absence of detailed exposure information, and the limitations of the epidemiologic tools with which metalworking fluid exposures have been studied. The evidence is unclear for an association between metalworking fluid exposure and cancer at several other sites including the stomach, esophagus, lung, prostate gland, brain, colon, and hematopoietic system.

Given the small number of epidemiologic studies that have adequate exposure characterization, the specific metalworking fluid constituents or contaminants responsible for the various site-specific cancer risks remain to be determined. The study with the most statistical power and detailed exposure information included more than 46,000 autoworkers in Michigan (Tolbert *et al*., 1992). Results of this study suggest that specific classes of metalworking fluids are associated with cancer at certain sites. However, within these metalworking fluid classes, the specific formulations responsible for the elevated cancer risks remain to be identified. Within the Tolbert *et al*. (1992) study, straight oil exposure was modestly associated with increased risks for laryngeal and rectal cancers, and there was limited evidence that synthetic metalworking fluid exposure was associated with an increased risk for pancreatic cancer. Subsequent case-control studies based on the original cohort have confirmed the association of laryngeal cancer with straight oil metalworking fluids (Eisen *et al*., 1994), and the association of pancreatic cancer with synthetic metalworking fluids (Bardin *et al*., 1997). The Tolbert *et al*. (1992) study found less evidence that soluble oil metal working fluid exposure is associated with cancer at any specific site. The most recent extension of this study up to 2005 added a follow-up for incident cancer. The cohort was limited to white males hired after 1938. The results of this evaluation provided evidence that oil-based metalworking fluids, especially straight mineral oils, were associated with an increased incidence of malignant melanoma (Costello *et al*., 2011). In another evaluation of the same incident cohort, long-term dermal exposure to oil-based metalworking fluids was reported to cause an increased risk of nonseminomatous testicular germ cell cancer (Langner *et al*., 2010; Behrens *et al*., 2012). An assessment of cancer incidence risk in these autoworkers exposed to metalworking fluids was published by Friesen *et al*. (2011). The findings supported the previously published work on this cohort and demonstrated that water-based metalworking fluids increased colon cancer risk and decreased stomach cancer risk.

The studies that provide most of the data suggesting an association between metalworking fluid exposure and cancer involved workers employed as early as the 1930s and as late as the mid-1980s. Because there is a latency period of 10 to 20 years between initial exposure to a carcinogen and the initial appearance of a solid-organ cancer caused by that carcinogen, the excess cancer mortality observed in these cohort studies most likely reflects the cancer risk associated with exposure conditions in the mid-1970s and earlier. Over the last several decades, substantial changes have been made in the metalworking industry, including changes in metalworking fluid composition, reduction of impurities, and reduction of exposure concentrations. These changes have likely reduced the cancer risks. However, since the epidemiologic data do not usually identify the metalworking fluid composition and impurities associated with the cancer risks observed in earlier cohorts, there is insufficient data to conclude that these changes will have eliminated all carcinogenic risks. The risk of cancer from metalworking fluid exposures in the mid-1970s and later remains to be determined because a definitive study has not yet been conducted on workers entering metalworking fluid-exposed jobs during this period. Thus, there is an unclear potential for current metalworking fluids to pose a similar carcinogenic hazard.

Genetic Toxicity

No studies were found in the literature on the genotoxicity of TRIM VX. Various components of TRIM VX were negative in bacterial mutagenicity tests conducted by the NTP, including TEA, myristic acid, oleic acid, stearic acid, diethylene glycol, diethylene glycol monobutyl ether, propylene glycol, and 4-chloro-3-methylphenol. α-Terpineol, another component of TRIM VX, exhibited weak activity in *Salmonella typhimurium* strain TA102 with and without rat liver S9 mix; no activity was observed in any of several other bacterial strains (Gomes-Carneiro *et al*., 1998). Genotoxicity information could not be found for another component of this metalworking fluid, palmitic acid.

STUDY RATIONALE

The class of metalworking fluids was nominated by NIOSH based on the large number of occupationally exposed workers, the associated epidemiologic data indicating increased incidences of laryngeal cancer in workers exposed to metalworking fluids, and the lack of carcinogenicity and chronic toxicology data for this class of complex mixtures. Three classes, including soluble oils, semisynthetic fluids, and synthetic fluids, encompass the majority of metalworking fluids in current use. The National Toxicology Program (NTP), in collaboration with NIOSH, selected metalworking fluids for inhalation toxicity testing by the NTP based on a combination of considerations including commercial availability, chemical composition, and class. TRIM VX, a soluble oil metalworking fluid, was selected for 3-month inhalation studies along with three other metalworking fluids (CIMSTAR 3800, Syntilo 1023, and TRIM SC210). TRIM VX was selected for 2-year studies based on the incidence of fibrosis of the lung; TRIM VX was the only metalworking fluid with this lesion following a 3-month exposure.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF TRIM® VX

TRIM® VX was obtained from Master Chemical Corporation (Perrysburg, OH) in two lots (101607N and 011509N). Lot 101607N was used during the 3-month studies, and lot 011509N was used during the 2-year studies. Characterization and stability analyses of the test material were conducted by the analytical chemistry laboratory at Chemir Analytical Services (Maryland Heights, MO) and by the study laboratory at Battelle Toxicology Northwest (Richland, WA) (Appendix H). Reports on analyses performed in support of the TRIM® VX studies are on file at the National Institute of Environmental Health Sciences.

The test material was a dark brown liquid. Fourier transform infrared (FTIR) spectroscopy was used to estimate the relative presence of various functional groups and create a reference for future comparisons of the same lot.

Analyses for both lots included Karl Fischer titration for water content; the determination of pH, specific gravity, and refractive index; elemental analysis for carbon, hydrogen, nitrogen, and sulfur using a C, H, N, S analyzer; metal analysis using inductively coupled plasma/optical emission spectrometry; chlorine, chloride, nitrate, and nitrite analysis by ion chromatography with conductivity detection; and iodine and iodide by ion-selective electrode titration. The study laboratory initially analyzed for general organic components using gas chromatography (GC) with flame ionization detection (FID) and identified the major components using GC with mass spectrometry (MS) (lot 101607N). Alkanolamines were identified and quantified using liquid chromatography (LC)/MS. Major oil constituents of both lots were assessed using GC/FID (lots 101607N and 011509N). The total amount of n-hexane extractable material and the amounts of bacteria and fungi were also determined. Samples were collected from the top, middle, and bottom of a drum and analyzed in triplicate to determine specific gravity, pH, refractive index, bacteria and fungi, total n-hexane extractable material and organic constituents.

The pH of the neat test material was approximately 7.5. In general, the FTIR spectra of the two lots were similar and were consistent with the presence of organic amines.

The test material did not contain significant amounts of water soluble nitrates, nitrites, chlorides, or iodides. The analysis for organics identified 12 compounds (Table 1). The amount of hexane extractable materials was 80.2% (lot 101607N) and 85.0% (lot 011509N). The mass balance percentages, estimated based on elemental contributions, resulted in 95% (lot 101607N) and 93% (lot 011509N) coverage. The composition of both lots was similar based on these analyses. The amount of bacteria and fungi was less than 100 CFU/mL and 10 CFU/mL, respectively, in samples diluted to 10% for analysis.

To ensure stability, the bulk test material was stored at room temperature, protected from light in metal drums. Periodic reanalyses of lot 011509N were performed by the analytical chemistry laboratory and the study laboratory at least every 6 months during the 2-year studies. FTIR spectra were obtained and compared to the reference spectrum of this lot obtained at the initial characterization; determinations were made for the pH, specific gravity, and refractive index; determination and quantitation of alkanolamines was performed using LC/MS; assessment of fatty acid methyl esters was performed using GC/FID; and the amounts of bacteria and fungi were determined. Based on these analyses, no degradation of the bulk test material was observed over the course of the study (Table H3). Furthermore, the amounts of bacteria and fungi were below the limit of detection of the assay (Table H3).

AEROSOL GENERATION AND EXPOSURE SYSTEM

The generation and delivery system used in the 3-month and 2-year studies consisted of two generator assemblies configured together so that the output from each assembly containing multi-jet nebulizers was directed to a common distribution line. TRIM® VX was continuously pumped to the liquid reservoir from the chemical cabinet

TABLE 1
Measured Components of the Two Lots of TRIM VX Used in the Inhalation Studies of TRIM VX[a]

Component	Lot 101607N[b]	Lot 011509N[c]
Water	7.1	6.8
Hexane extractable material	80.2	85.0
Identified organic compounds		
Triethanolamine	3.7	3.2
4-Chloro-3-methyl-phenol	3.59	2.49
Diethylene glycol	0.87	1.07
Diethylene glycol monobutyl ether	1.02	1.11
Methyl palmitate	1.18	1.20
Methyl oleate	5.65	5.81
Methyl stearate	0.89	0.93
Myristic acid	0.49	0.23
Oleic acid	3.18	1.23
Palmitic acid	1.01	0.31
Propylene glycol	0.20	0.20
α-Terpineol	0.60	0.50

[a] All values are percentages
[b] Used in the 3-month studies
[c] Used in the 2-year studies

reservoir by metering pumps during the aerosol generation process. Ports in the generator assembly introduced compressed air to drive the nebulizers and dilution air to transport aerosol to the distribution line.

High velocity compressed air created a vacuum in the liquid uptake tube that drew test material from the liquid reservoir into the multi-jet nebulizer streams where shear forces broke the resultant liquid filaments into droplets. Large droplets were impacted on the impaction plate of the nebulizer or the generator assembly walls and were returned to the liquid reservoir; smaller droplets were drawn into the dilution air and transported to the common distribution line. The common distribution line was divided into two branches to supply aerosol to exposure chambers located on both sides of the exposure room; each branch line terminated in a filter protecting the flowmeter controlling the line via the house vacuum supply. During exposures, at each chamber position, aerosol was removed from the distribution line and injected into a tee fitting where it was directed to the inlet of the exposure chamber where it was mixed with conditioned air to achieve the desired exposure concentrations.

AEROSOL CONCENTRATION MONITORING

Summaries of the chamber aerosol concentrations are given in Tables H3 and H4. The concentrations of methyl palmitate, methyl stearate, and methyl oleate in TRIM® VX were monitored using GC/FID and compared to real-time aerosol monitor (RAM) measurements. The monitors were connected to the chambers by a sampling system designed by Battelle incorporating a valve that multiplexed each RAM to a 0 mg/m^3 chamber or the room, a HEPA-filtered room air blank, and two additional exposure chambers. The output voltage of the RAM was recorded by a program designed by Battelle (Battelle Exposure Data Acquisition and Control) to select the correct sample stream and acquire a raw voltage signal from each RAM. Equations for the calibration curves resided within the program and were used to convert the measured RAM voltages to exposure chamber concentrations. Each RAM was calibrated by constructing a response curve using the measured RAM voltages (voltage readings were corrected by subtracting the RAM zero-offset voltage from measured RAM voltages) and concentrations of methyl palmitate, methyl stearate, and methyl oleate in TRIM® VX that were determined by analyzing duplicate adsorbent gas sampling tubes collected daily from the exposure chambers.

For the 3-month and 2-year studies, methyl palmitate, methyl stearate, and methyl oleate in TRIM® VX were extracted from the gas sampling tubes and analyzed using GC/FID. The GC/FID instrument was calibrated against serially diluted TRIM® VX and the internal standard, methyl undecanoate.

Chamber Atmosphere Characterization

Particle size distribution in each chamber was determined prior to the start of all studies and monthly during the studies. Impactor samples were taken from each exposure chamber using a Mercer-style seven-stage impactor and the stages were collected on glass coverslips lightly coated with silicone (stages 1-7) or filters (stage 8) and were analyzed by GC/FID for methyl oleate as a marker for TRIM® VX. The relative mass collected on each stage was analyzed by the CASPACT impactor analysis program developed at Battelle based on probit analysis (Hill *et al*., 1977). The resulting estimates of the mass median aerodynamic diameter (range 1.7 to 2.2 µm) and the geometric standard deviation (range 1.7 to 1.9 µm) of each set of samples are given in Tables H5 through H7.

Buildup and decay rates for chamber aerosol concentrations were determined with and without animals present in the chambers. At a chamber air flow rate of 15 cubic feet per minute, the theoretical value for the time to achieve 90% of the target concentration after the beginning of aerosol generation (T_{90}) was approximately 9.2 minutes. T_{90} values of 10 minutes and 12 minutes were selected for the 3-month and 2-year studies, respectively.

The uniformity of aerosol concentration in the inhalation exposure chambers was measured once during the 3-month studies with animals present, once before the 2-year studies began without animals present, and approximately every three months during the 2-year studies with animals present. RAM measurements were taken from 12 different chamber positions. Chamber concentration uniformity was acceptable throughout the 3-month and 2-year studies (Appendix H).

The persistence of TRIM® VX in the exposure chambers was monitored after aerosol delivery ended by monitoring the concentration in the 400 mg/m^3 chambers in the 3-month studies with animals present and in the 100 mg/m^3 chambers in the 2-year studies with and without animals present. In the 3-month studies, the concentration decreased to 1% of the starting concentration within approximately 18 minutes. In the 2-year study of male rats, the concentration decreased to 1% of the starting concentration within approximately 20 minutes with animals present and within 17 minutes without animals. In the 2-year studies of female rats and male and female mice, the concentration decreased to 1% of the starting concentration within 17 minutes with animals present and within 15 minutes without animals.

Stability studies of the test material in the generation and exposure system were performed before (2-year studies only) and during the studies by the study laboratory. Samples were collected, prepared, and assayed for oil constituents using GC/FID or LC/MS. The relative amounts of the major constituents in the samples generally reflected those of the bulk test material with the exceptions of propylene glycol, diethylene glycol, diethylene glycol monobutyl ether, and α-terpineol.

Animal Source

Male and female Wistar Han [Crl:WI (Han)] rats, referred to as Wistar Han rats in this Technical Report, were obtained from Charles River Laboratories (Raleigh, NC). Male and female B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc. (Germantown, NY).

Animal Welfare

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Studies were approved by the Battelle Toxicology Northwest Animal Care and Use Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

3-Month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to TRIM VX and to determine the appropriate exposure concentrations to be used in the 2-year studies.

On receipt, the rats were 4 weeks old and mice were 4 to 5 weeks old. Animals were quarantined for 12 days and were 6 weeks (rats) or 5 to 6 weeks (mice) old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. The health of the animals was monitored during the studies according to using the protocols of the NTP Sentinel Animal Program (Appendix J). All test results were negative.

Groups of 10 male and 10 female rats and mice were exposed by whole body inhalation to TRIM VX aerosol at concentrations of 0, 25, 50, 100, 200, or 400 mg/m^3, 6 hours plus T_{90} (10 minutes) per day, 5 days per week for 14 weeks. Exposure concentrations for the 3-month studies were selected based on results from previously conducted 3-month toxicity studies in rats and mice. The highest achievable concentration in the 3-month studies was 400 mg/m^3 and minimal toxicity was reported. Thus, 400 mg/m^3 was selected as the highest exposure concentration for the 3-month studies in rats and mice. Feed was available *ad libitum* except during exposure periods; water was available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded after exposure on day 1, twice daily on days 2 through 5, 8 through 12, 15, and 19, weekly thereafter, and at study termination. The animals were weighed initially, on days 6, 13, and 19, weekly thereafter, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

Blood was collected from the retroorbital plexus of rats and the retroorbital sinus of mice at the end of the studies for hematology and clinical chemistry (rats only) analyses. For the hematology samples, blood was collected in tubes containing potassium EDTA; for the clinical chemistry samples, the blood was collected in a tube devoid of anticoagulant but containing a separator gel for serum. An ADVIA 120 (Siemans Medical Solutions Diagnostics, Tarrytown, NY) was used to determine packed cell volume; hemoglobin concentration; erythrocyte, reticulocyte, platelet, and leukocyte counts; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration. Manual hematocrit values were determined using a microcentrifuge (Heraeus Haemofuge, Heraeus Holding GmbH; Hanau, Germany) and a Damon/IEC capillary reader (International Equipment Company, Needham Heights, MA) for comparison to ADVIA 120 values for packed cell volume. Blood smears were stained with a Romanosky-type aqueous stain in a Wescor 7120 slide stainer (Wescor, Inc., Logan, UT). Leukocyte differential counts were based on classifying a minimum of 100 white cells. Blood samples for clinical chemistry analyses were analyzed using a Roche Hitachi 912 System (Roche Diagnostic Corporation, Indianapolis, IN). The hematology and clinical chemistry parameters measured are listed in Table 2.

Necropsies were performed on all animals. The heart, right kidney, liver, lungs, spleen, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution, and testes with vaginal tunics and epididymides were first fixed in a modified Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed by the study laboratory pathologist on chamber control and 400 mg/m^3 groups of rats and mice. Table 2 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats and mice were exposed by whole body inhalation to TRIM VX aerosol at concentrations of 0, 10, 30, or 100 mg/m^3, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks. Rats and mice were quarantined for 12 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats were approximately 6 weeks old and mice approximately 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix J). All test results were negative.

Rats and mice were housed individually. Feed was available *ad libitum* except during exposure periods; water was available *ad libitum*. Cages were rotated weekly. Further details of animal maintenance are given in Table 2. The feed was analyzed for contaminants and found acceptable. Information on feed composition and contaminants is provided in Appendix I.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded at week 5, every 4 weeks thereafter, and at study termination. Body weights were recorded on day 1, weekly for 13 weeks, every 4 weeks thereafter, and at study termination.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution, and testes with vaginal tunics and epididymides were first fixed in modified Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 2.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent QA laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a QA pathologist evaluated slides from all tumors and all potential target organs, which included the lung, larynx, and nose of rats and mice and the bronchial lymph node and liver of mice.

The QA report and the reviewed slides were submitted to the NTP PWG coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and QA pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and QA pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the QA pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

Formalin-fixed lung tissue from chamber control and 100 mg/m^3 male and female rats from the 2-year study were retrieved from the NTP Archives, passed through a graded series of sucrose solutions (10%, 20%, and 30%, successively) to remove water from the tissues, and then frozen in Optimum Cutting Temperature medium. The frozen lung tissue was cryosectioned at 5 µm, and the cryosections were stained with Oil-Red-O histochemical stain, a fat-soluble diazo dye, to demonstrate oil, fat, or lipids in the tissues.

TABLE 2
Experimental Design and Materials and Methods in the Inhalation Studies of TRIM VX

3-Month Studies	2-Year Studies
Study Laboratory	
Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)
Strain and Species	
Wistar Han [Crl:WI (Han)] rats B6C3F1/N mice	Wistar Han [Crl:WI (Han)] rats B6C3F1/N mice
Animal Source	
Rats: Charles River Laboratories (Raleigh, NC) Mice: Taconic Farms, Inc. (Germantown, NY)	Rats: Charles River Laboratories (Raleigh, NC) Mice: Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
12 days	12 days
Average Age When Studies Began	
Rats: 6 weeks Mice: 5 to 6 weeks	Rats: 6 weeks Mice: 5 to 6 weeks
Date of First Exposure	
July 14, 2008	Rats: July 20, 2009 Mice: August 3, 2009
Duration of Exposure	
6 hours plus T_{90} (10 minutes) per day, 5 days per week, for 14 weeks	6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 105 weeks
Date of Last Exposure	
Rats: October 13 (males) or 14 (females), 2008 Mice: October 15 (males) or 16 (females), 2008	Rats: July 17-19 (males) or 19-21 (females), 2011 Mice: July 31-August 2 (males) or August 2-4 (females), 2011
Necropsy Dates	
Rats: October 14 (males) or 15 (females), 2008 Mice: October 16 (males) or 17 (females), 2008	Rats: July 18-20 (males) or 20-22 (females), 2011 Mice: August 1-3 (males) or 3-5 (females), 2011
Average Age at Necropsy	
Rats: 19 weeks Mice: 19 to 20 weeks	Rats: 110 to 111 weeks Mice: 109 to 111 weeks
Size of Study Groups	
10 males and 10 females	50 males and 50 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month studies
Animals per Cage	
1	1
Method of Animal Identification	
Tail tattoo	Tail tattoo
Diet	
Irradiated NTP-2000 wafers (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, except during exposure periods, changed weekly	Same as 3-month studies

TABLE 2
Experimental Design and Materials and Methods in the Inhalation Studies of TRIM VX

3-Month Studies	2-Year Studies
Water Tap water (Richland, WA, municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 3-month studies
Cages Stainless steel wire bottom (Lab Products, Inc., Seaford, DE), changed weekly, rotated daily on exposure days through day 15 then weekly within chambers	Same as 3-month studies, except rotated weekly within chambers
Cageboard Techboard® Ultra untreated paper excreta pan liner (Shepherd Specialty Papers, Watertown, TN), changed daily	Same as 3-month studies
Chamber Air Supply Filters Single HEPA (open stock) charcoal (RSE, Inc., New Baltimore, MI) Purafil (Environmental Systems, Lynnwood, WA), all new at study start	Same as 3-month studies, except HEPA changed annually
Chambers Stainless steel, excreta pan at each of six levels (Lab Products, Inc., Seaford, DE), changed weekly, excreta pans changed daily	Same as 3-month studies
Chamber Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15 ± 2/hour	Same as 3-month studies
Exposure Concentrations 0, 25, 50, 100, 200, and 400 mg/m^3	0, 10, 30, and 100 mg/m^3
Type and Frequency of Observation Observed twice daily; animals were weighed initially, on days 6, 13, and 19, weekly thereafter, and at the end of the studies; clinical findings were recorded on day 1 postexposure, twice daily on days 2 through 5, 8 through 12, 15, and 19, weekly thereafter, and at the end of the studies.	Observed twice daily; animals were weighed initially, weekly for 13 weeks, monthly thereafter, and at the end of the studies; clinical findings were recorded at week 5, monthly thereafter, and at the end of the studies.
Method of Kill Carbon dioxide asphyxiation	Same as 3-month studies
Necropsy Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, spleen, right testis, and thymus.	Necropsies were performed on all animals.
Clinical Pathology Blood was collected from the retroorbital plexus of rats and the retroorbital sinus of mice at the end of the studies for hematology and clinical chemistry (rats only). ***Hematology:*** hematocrit; packed cell volume; hemoglobin; erythrocyte, reticulocyte, nucleated erythrocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte counts and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, glucose, total protein, albumin, globulin, cholesterol, triglycerides, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and total bile acids	None

TABLE 2
Experimental Design and Materials and Methods in the Inhalation Studies of TRIM VX

3-Month Studies	2-Year Studies
Histopathology Complete histopathology was performed on chamber control and 400 mg/m^3 rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung (with bronchus), lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus.	Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus.

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B4, C1, C4, D1, and D4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal kill.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal kill; if the animal died prior to terminal kill and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344/N rats and B6C3F1/N mice (Portier *et al.*, 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology and clinical chemistry data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical control database must be generally similar. Significant factors affecting the background incidences of neoplasms at a variety of sites are diet, sex, strain/stock, and route of exposure. The NTP historical control database contains all 2-year studies for each species, sex, and strain/stock with histopathology findings in control animals completed within the most recent 5-year period (Haseman, 1992, 1995; Haseman and Rao, 1992). In general, the historical control database for a given study includes studies using the same route of administration, and the overall incidences of neoplasms in controls for all routes of administration are included for comparison, including the current study.

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 3-month and 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent QA contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of TRIM VX was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* and increases in the frequency of micronucleated erythrocytes in rat and mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies (Witt *et al.*, 2000). Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

RESULTS

RATS

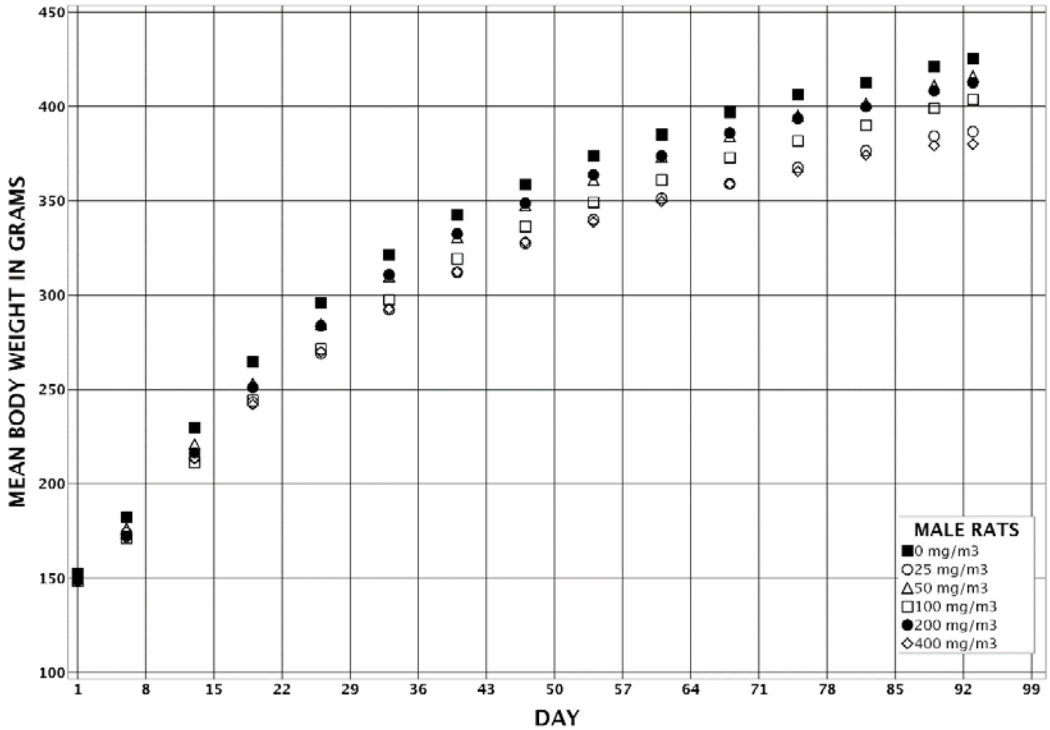
3-MONTH STUDY

All rats survived to the end of the study, and the mean body weights of exposed groups of females were similar to those of the chamber controls (Table 3; Figure 1). Final mean body weights of 25 and 400 mg/m^3 males and the mean body weight gain of 400 mg/m^3 males were significantly less than those of the chamber controls; however, the effect in the 25 mg/m^3 males was not considered biologically relevant. There were no chemical-related clinical observations in male or female rats (NTP, 2016a).

TABLE 3
Survival and Body Weights of Rats in the 3-Month Inhalation Study of TRIM VX[a]

Concentration (mg/m^3)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	152 ± 3	425 ± 8	273 ± 8	
25	10/10	149 ± 3	387 ± 8*	238 ± 7	91
50	10/10	150 ± 3	416 ± 9	267 ± 10	98
100	10/10	148 ± 3	404 ± 8	255 ± 8	95
200	10/10	149 ± 2	413 ± 12	263 ± 12	97
400	10/10	150 ± 3	380 ± 14*	230 ± 13*	89
Female					
0	10/10	117 ± 3	237 ± 6	120 ± 5	
25	10/10	116 ± 2	222 ± 5	106 ± 5	94
50	10/10	115 ± 2	230 ± 5	115 ± 5	97
100	10/10	115 ± 2	232 ± 6	118 ± 4	98
200	10/10	117 ± 3	236 ± 6	119 ± 7	99
400	10/10	116 ± 2	227 ± 6	111 ± 5	96

* Significantly different ($P \leq 0.05$) from the chamber control group by Dunnett's test
[a] Weights and weight changes are given as mean ± standard error.
[b] Number of animals surviving at 14 weeks/number initially in group



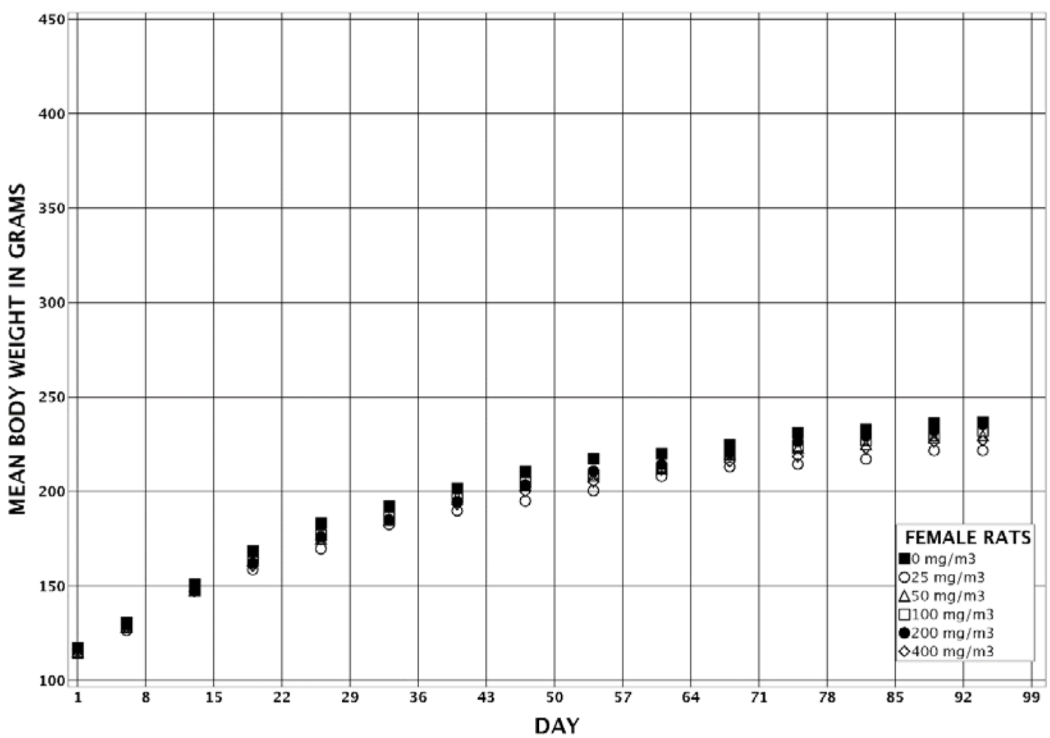


FIGURE 1
Growth Curves for Rats Exposed to TRIM VX by Inhalation for 3 Months

A low number of changes were observed in the hematology parameters; however, these changes were mild and not considered toxicologically relevant (Table F1). Several minimal to mild or sporadic alterations in the clinical chemistry parameters were also observed, but were not considered toxicologically relevant (Table F1).

The relative liver weights of 200 and 400 mg/m^3 males and the absolute and relative liver weights of 200 and 400 mg/m^3 females were significantly increased by 8% to 18% compared to those of the chamber controls (Tables 4 and G1). There were no histopathologic changes in liver corresponding to weight changes. The absolute and relative lung weights of females exposed to 100 mg/m^3 or greater were significantly increased (up to 20%) compared to those of the chamber controls. The absolute and relative lung weights of all exposed male groups were similar to those of the chamber controls.

In the lung, there were significantly increased incidences of fibrosis and chronic active inflammation in males and females exposed to 50 mg/m^3 or greater and of histiocytic cellular infiltration in males and females exposed to 100 mg/m^3 or greater (Table 5). Fibrosis was characterized by the expansion of alveolar ducts or septae by collagen and fibrocytes, mainly at the junction of alveolar ducts and alveoli or at the junction of terminal bronchioles and alveolar ducts. The changes were mild in the 400 mg/m^3 group and minimal in the other exposed groups. Fibrosis was confirmed by Masson's trichrome staining of selected lung slides. Chronic active inflammation in the lung was composed of a few lymphocytes, macrophages, or rare neutrophils in the areas of fibrosis and this change was graded as minimal in severity. When the inflammatory cells were also present in the peribronchiolar or perivascular interstitium, the change was graded as mild in severity. Histiocytic cellular infiltration was characterized by the presence of minimal to mild increases in vacuolated alveolar macrophages diffusely scattered within the alveolar spaces. Many of the macrophages were large and contained one to many, clear, round to oval cytoplasmic vacuoles; some also contained brown granular material.

TABLE 4
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Inhalation Study of TRIM VX[a]

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
n	10	10	10	10	10	10
Male						
Necropsy body wt	425 ± 8	387 ± 8*	416 ± 9	404 ± 8	413 ± 12	380 ± 14*
Liver						
Absolute	12.30 ± 0.35	11.75 ± 0.25	12.52 ± 0.30	12.18 ± 0.47	12.87 ± 0.39	12.59 ± 0.73
Relative	28.903 ± 0.499	30.416 ± 0.370	30.125 ± 0.452	30.101 ± 0.785	31.244 ± 0.645*	33.002 ± 0.935**
Lung						
Absolute	2.50 ± 0.13	2.17 ± 0.11	2.44 ± 0.16	2.44 ± 0.14	2.52 ± 0.08	2.54 ± 0.12
Relative	5.893 ± 0.311	5.622 ± 0.294	5.890 ± 0.430	6.048 ± 0.312	6.154 ± 0.250	6.715 ± 0.334
Female						
Necropsy body wt	237 ± 6	222 ± 5	230 ± 5	232 ± 6	236 ± 6	227 ± 6
Liver						
Absolute	7.39 ± 0.21	6.95 ± 0.25	7.14 ± 0.25	7.56 ± 0.15	8.25 ± 0.36*	8.37 ± 0.25**
Relative	31.311 ± 0.855	31.326 ± 0.758	31.045 ± 0.450	32.658 ± 0.620	34.940 ± 0.888**	36.843 ± 0.390**
Lung						
Absolute	1.60 ± 0.08	1.40 ± 0.06	1.56 ± 0.07	1.85 ± 0.11*	1.86 ± 0.08*	1.85 ± 0.06*
Relative	6.779 ± 0.279	6.301 ± 0.182	6.778 ± 0.199	7.930 ± 0.368**	7.953 ± 0.378**	8.157 ± 0.246**

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' or Dunnett's test
** Significantly different ($P \leq 0.01$) from the chamber control by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE 5
Incidences of Nonneoplastic Lesions of the Respiratory System in Rats in the 3-Month Inhalation Study of TRIM VX

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
Male						
Lung[a]	10	10	10	10	10	10
Fibrosis[b]	0	0	10** (1.0)[c]	10** (1.0)	10** (1.1)	10** (2.0)
Infiltration Cellular, Histiocyte	0	0	1 (1.0)	10** (1.2)	10** (2.0)	10** (2.0)
Inflammation, Chronic Active	0	0	8** (1.0)	10** (1.0)	10** (1.1)	10** (2.0)
Nose	10	10	10	10	10	10
Goblet Cell, Hyperplasia	0	0	1 (1.0)	3 (1.0)	10** (1.0)	8** (1.0)
Inflammation, Suppurative	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)
Olfactory Epithelium, Accumulation, Hyaline Droplet	1 (1.0)	10** (1.7)	10** (2.0)	10** (2.3)	10** (2.7)	10** (2.5)
Respiratory Epithelium, Accumulation, Hyaline Droplet	0	10** (1.7)	10** (2.0)	10** (2.3)	10** (2.7)	7** (2.3)
Respiratory Epithelium, Hyperplasia	0	0	0	0	9** (1.0)	10** (1.0)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	4* (1.0)	7** (1.0)
Larynx	10	10	10	10	10	10
Hyperplasia, Squamous	0	8** (1.0)	8** (1.4)	7** (1.0)	10** (1.5)	10** (2.4)
Inflammation, Chronic Active	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)
Metaplasia, Squamous	0	10** (2.1)	10** (2.5)	10** (2.5)	10** (3.4)	10** (3.4)
Female						
Lung	10	10	10	10	10	10
Fibrosis	0	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (2.0)
Infiltration Cellular, Histiocyte	0	0	1 (1.0)	10** (1.3)	10** (1.9)	10** (2.0)
Inflammation, Chronic Active	3 (1.0)	0	9** (1.1)	10** (1.0)	10** (1.1)	10** (2.0)
Nose	10	10	10	10	10	10
Goblet Cell, Hyperplasia	0	0	0	3 (1.0)	9** (1.0)	9** (1.0)
Inflammation, Suppurative	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)
Olfactory Epithelium, Accumulation, Hyaline Droplet	0	10** (2.0)	10** (2.4)	10** (2.6)	10** (2.6)	10** (2.6)
Respiratory Epithelium, Accumulation, Hyaline Droplet	0	10** (2.0)	9** (2.4)	10** (2.6)	10** (2.6)	10** (2.6)
Respiratory Epithelium, Hyperplasia	0	0	0	1 (1.0)	10** (1.0)	10** (1.0)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	2 (1.0)	5* (1.0)	7** (1.0)
Larynx	10	10	10	10	10	10
Hyperplasia, Squamous	0	8** (1.0)	4* (1.0)	7** (1.4)	8** (1.5)	10** (1.8)
Inflammation, Chronic Active	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.0)
Metaplasia, Squamous	0	10** (2.0)	10** (2.1)	10** (2.7)	10** (3.1)	10** (3.4)

* Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

In the nose, there were significantly increased incidences of suppurative inflammation and olfactory and respiratory epithelium hyaline droplet accumulation in all exposed groups of males and females (Table 5). There were also significantly increased incidences of goblet cell and respiratory epithelium hyperplasia and squamous metaplasia in 200 and 400 mg/m^3 males and females. Suppurative inflammation was minimal and consisted of small numbers of neutrophils in the submucosa subjacent to areas with hyaline droplet accumulation. Goblet cell hyperplasia consisted of minimally increased numbers of large goblet cells on the medial aspects of the maxilloturbinates of the Level I nasal section and in respiratory epithelium of the Level II nasal section. Hyaline droplet accumulation in the respiratory and olfactory epithelium lining the ethmoturbinates of the Level III nasal section was mild to moderate in severity and was characterized by the presence of variable-sized eosinophilic globules in the cytoplasm. Occasionally, the change was present in the epithelia of Level II. Respiratory epithelial hyperplasia was minimal, present in the ventral aspect of Level I, lateral to the vomeronasal organ at the junction of the squamous and respiratory epithelium, and was characterized by a four to five cell thick pseudostratified columnar epithelium. The change was also present in Level II and sometimes was associated with goblet cell hyperplasia. Squamous metaplasia of respiratory epithelium was of minimal severity and present at the tips of the naso- and maxillary turbinates and lateral walls in Level I. The features ranged from flattening or attenuation of cuboidal cells with loss of cilia to increased numbers of pseudostratified/stratified layers of cells.

In the larynx, there were significantly increased incidences of squamous hyperplasia, squamous metaplasia, and chronic active inflammation in all exposed groups of male and female rats (Table 5). Squamous hyperplasia in the larynx was characterized by thickening of the epithelium (more than three cell layers versus one to two cell layers in chamber controls) overlying the arytenoid cartilages. Squamous metaplasia involved the ventral epithelium at the base of the epiglottis, lateral walls of the anterior section (Level I), and dorsolateral walls and ventral diverticulum of the middle section (Level II). In these areas, the normally pseudostratified ciliated columnar epithelium lining the base of the epiglottis and the nonciliated cuboidal epithelium lining the ventral diverticulum were replaced by squamous epithelium which was sometimes keratinized. Frequently, the metaplastic squamous epithelium was also hyperplastic. Chronic active inflammation frequently accompanied squamous metaplasia and was characterized by the presence of small numbers of lymphocytes, plasma cells, macrophages, and neutrophils or eosinophils between the basal epithelium and the epiglottic cartilage or between the epithelial cells of the mucosa.

Exposure Concentration Selection Rationale: The exposure concentrations selected for the 2-year inhalation study in male and female Wistar Han rats were 10, 30, and 100 mg/m^3. The highest exposure concentration was based on the incidence and severity of lung fibrosis in the current 3-month study. Although minimal lung fibrosis was present in rats exposed to 50 and 100 mg/m^3, this lesion was not expected to affect survival in the 2-year study, and use of the same exposure concentrations for rats and mice would facilitate inter-species comparisons. In addition, these concentrations were used in the 2-year study of CIMSTAR 3800 in Wistar Han rats, which allows for comparisons between the two metalworking fluid studies.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 6 and in the Kaplan-Meier survival curves (Figure 2). Survival of all exposed groups of male and female rats was similar to that of the chamber control groups.

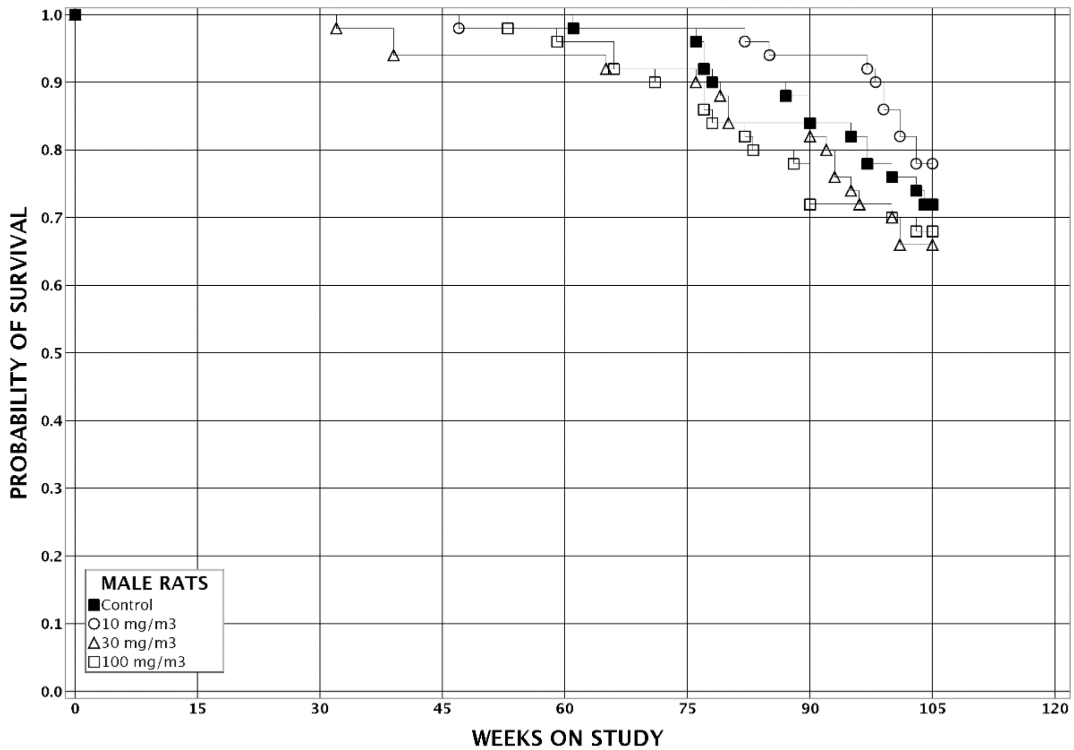
Body Weights and Clinical Findings

The mean body weights of all exposed groups of males and females were similar to those of the chamber control groups throughout the study (Tables 7 and 8; Figure 3). Clinical observations included abnormal breathing in a very few males and females exposed to 30 or 100 mg/m^3 (NTP, 2016b).

TABLE 6
Survival of Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Male				
Animals initially in study	50	50	50	50
Moribund	12	10	14	15
Natural deaths	2	1	3	1
Animals surviving to study termination	36	39	33	34
Percent probability of survival at end of study[a]	72	78	66	68
Mean survival (days)[b]	696	710	669	673
Survival analysis[c]	P=0.386	P=0.555N	P=0.588	P=0.686
Female				
Animals initially in study	50	50	50	50
Moribund	19	15	15	16
Natural deaths	1	2	2	4
Animals surviving to study termination	30[d]	33[d]	33	30
Percent probability of survival at end of study	60	66	66	60
Mean survival (days)	681	674	697	695
Survival analysis	P=1.000	P=0.680N	P=0.568N	P=0.947N

a Kaplan-Meier determinations
b Mean of all deaths (uncensored, censored, and terminal kill)
c The result of the life table trend test (Tarone, 1975) is in the chamber control column, and the results of the life table pairwise comparisons (Cox, 1972) with the chamber controls are in the exposed group columns. A lower mortality in an exposure group is indicated by **N**.
d Includes one animal that died during the last week of the study



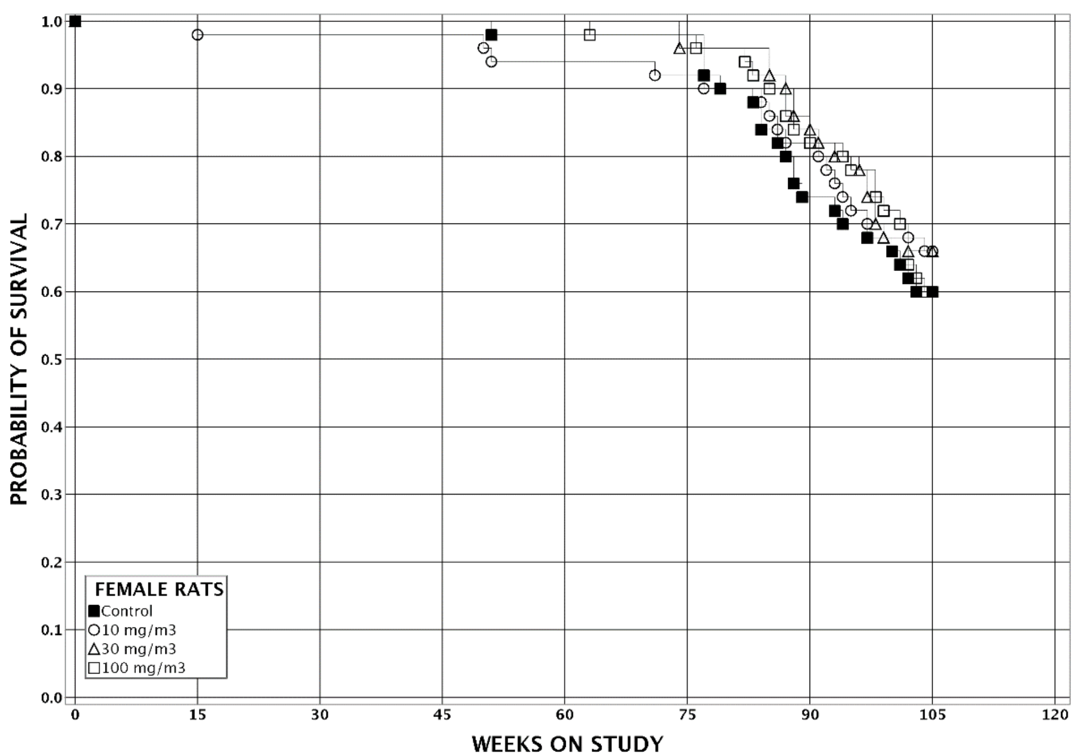


FIGURE 2
Kaplan-Meier Survival Curves for Rats Exposed to TRIM VX by Inhalation for 2 Years

TABLE 7
Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control		10 mg/m³			30 mg/m³			100 mg/m³		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	129	50	128	99	50	127	99	50	127	98	50
9	176	50	172	98	50	167	95	50	169	96	50
16	218	50	213	98	50	211	97	50	212	97	50
23	253	50	246	97	50	246	97	50	247	98	50
30	283	50	277	98	50	277	98	50	279	98	50
37	308	50	299	97	50	301	98	50	302	98	50
44	328	50	318	97	50	321	98	50	321	98	50
51	346	50	334	97	50	340	98	50	339	98	50
58	361	50	347	96	50	352	98	50	351	97	50
65	375	50	359	96	50	367	98	50	363	97	50
72	388	50	369	95	50	378	98	50	373	96	50
79	396	50	379	96	50	388	98	50	383	97	50
86	406	50	386	95	50	397	98	50	391	96	50
114	431	50	408	95	50	421	98	50	415	96	50
142	452	50	427	94	50	441	98	50	435	96	50
170	466	50	442	95	50	456	98	50	451	97	50
198	481	50	453	94	50	470	98	50	461	96	50
226	497	50	467	94	50	484	97	49	478	96	50
254	512	50	482	94	50	499	97	49	492	96	50
282	521	50	490	94	50	508	97	47	501	96	50
310	534	50	500	94	50	519	97	47	513	96	50
338	544	50	506	93	49	529	97	47	519	95	50
367	554	50	518	93	49	540	98	47	529	96	49
394	563	50	525	93	49	549	98	47	535	95	49
422	573	49	534	93	49	557	97	47	543	95	48
450	580	49	538	93	49	559	96	47	546	94	48
478	590	49	546	93	49	567	96	46	555	94	46
506	596	49	558	94	49	575	96	46	566	95	45
534	603	48	568	94	49	583	97	45	568	94	45
562	609	45	574	94	49	589	97	42	569	93	42
590	614	45	582	95	47	592	96	42	570	93	40
618	621	44	588	95	47	596	96	42	573	92	39
646	622	42	589	95	47	591	95	38	576	93	36
674	619	40	587	95	46	605	98	36	582	94	36
703	600	38	591	99	42	605	101	35	578	96	35
Mean for Weeks											
1-13	305		294	96		298	98		297	97	
14-52	493		464	94		481	98		474	96	
53-101	596		561	94		578	97		561	94	

TABLE 8
Mean Body Weights and Survival of Female Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control		10 mg/m^3			30 mg/m^3			100 mg/m^3		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	115	50	115	100	50	115	100	50	114	99	50
9	138	50	139	101	50	139	101	50	138	100	50
16	154	50	156	101	50	155	101	50	155	100	50
23	168	50	170	101	50	171	101	50	169	100	50
30	179	50	181	101	50	180	101	50	180	100	50
37	188	50	190	101	50	191	102	50	191	101	50
44	197	50	199	101	50	199	101	50	198	101	50
51	205	50	206	101	50	206	101	50	204	99	50
58	209	50	213	102	50	212	101	50	211	101	50
65	215	50	218	101	50	218	101	50	216	101	50
72	221	50	222	100	50	222	100	50	220	100	50
79	224	50	226	101	50	226	101	50	223	100	50
86	227	50	228	101	50	227	100	50	226	100	50
114	239	50	241	101	49	239	100	50	240	100	50
142	246	50	251	102	49	246	100	50	246	100	50
170	256	50	258	101	49	251	98	50	252	99	50
198	260	50	264	102	49	259	100	50	256	99	50
226	267	50	273	102	49	264	99	50	265	99	50
254	275	50	276	101	49	271	99	50	270	98	50
282	279	50	283	102	49	277	99	50	277	99	50
310	289	50	296	103	49	288	100	50	285	99	50
338	296	50	299	101	49	294	99	50	291	98	50
367	301	49	313	104	47	303	101	50	300	100	50
394	313	49	322	103	47	312	100	50	307	98	50
422	324	49	331	102	47	324	100	50	318	98	50
450	334	49	341	102	47	333	100	50	321	96	49
478	342	49	352	103	47	342	100	50	329	96	49
506	353	49	362	103	46	353	100	50	339	96	49
534	359	48	374	104	45	361	101	48	351	98	48
562	361	45	381	106	45	365	101	48	355	98	48
590	367	42	393	107	43	373	102	47	361	98	46
618	372	37	402	108	41	380	102	43	368	99	42
646	378	36	411	109	39	381	101	40	368	98	41
674	389	34	420	108	35	388	100	38	372	96	39
703	391	32	424	108	35	391	100	34	371	95	36
Mean for Weeks											
1-13	188		189	101		189	101		188	100	
14-52	267		271	101		265	99		265	99	
53-101	352		371	105		354	100		343	97	

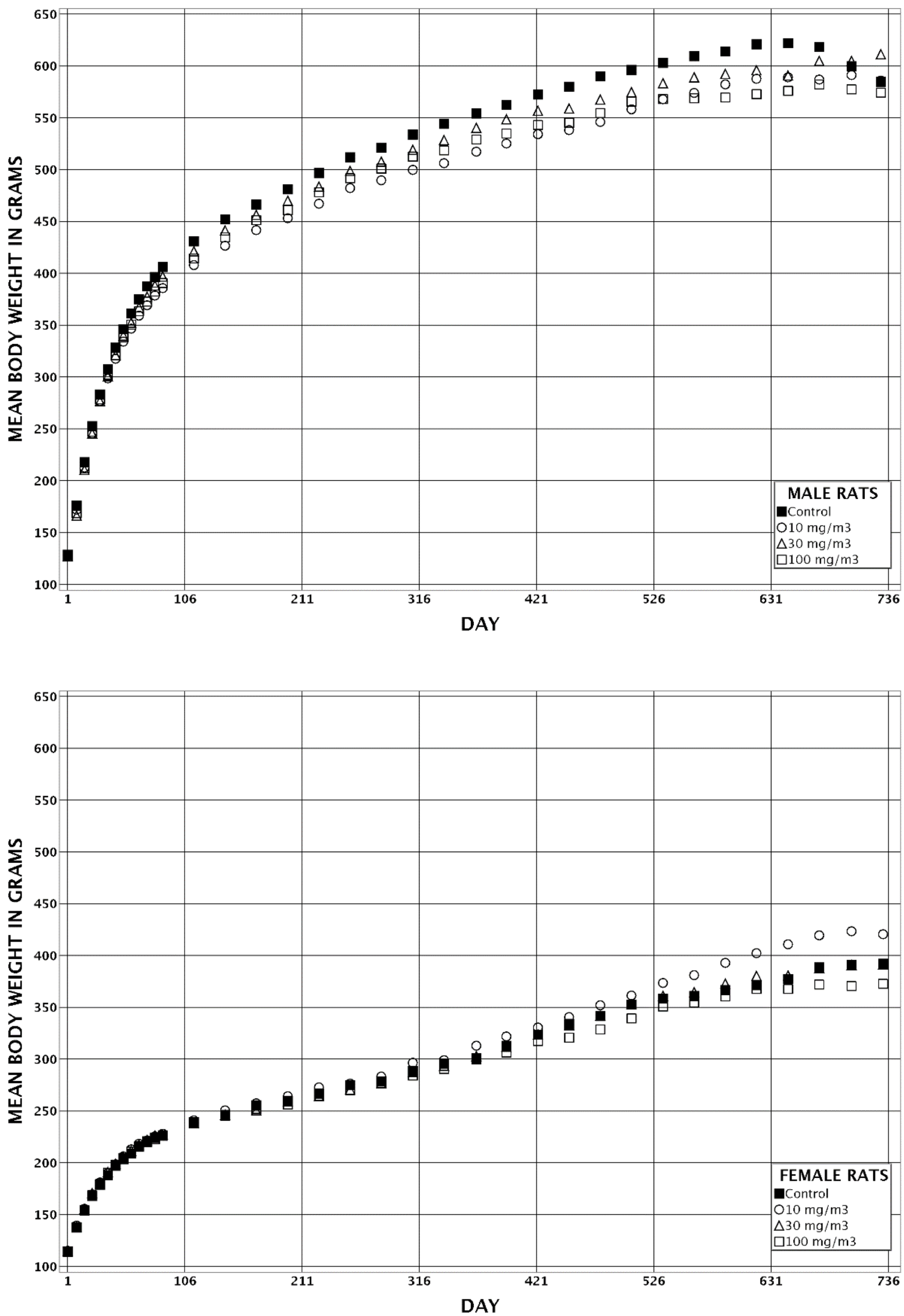


FIGURE 3
Growth Curves for Rats Exposed to TRIM VX by Inhalation for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the lung, nose, larynx, and bronchial and mediastinal lymph nodes. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Lung: There was an increased incidence of alveolar/bronchiolar carcinoma in 100 mg/m^3 males that resulted in a positive trend for carcinoma alone (Tables 9, A1, and A2). When combined with the incidence of alveolar/bronchiolar adenoma in 100 mg/m^3 males, there was a positive trend in the incidences; however, the combined incidence was not statistically significant compared to the chamber control group. In females, there was an increased incidence of alveolar/bronchiolar adenoma at 100 mg/m^3 which included two animals with multiple adenomas (Tables 9, B1, and B2). Although the incidence of alveolar/bronchiolar adenoma in the 100 mg/m^3 females was not statistically significant, there was a positive trend in the incidences of this neoplasm. Alveolar/bronchiolar carcinomas in males and adenomas in females have not occurred in historical control Wistar Han rats (Tables 9, A3, and B3). One 100 mg/m^3 female had a cystic keratinizing epithelioma; this neoplasm has not occurred in historical controls from all routes of exposure (Tables 9 and B3).

Alveolar/bronchiolar neoplasms were morphologically typical of those that occur spontaneously in rats. Adenomas were small, circumscribed, and nodular to slightly irregularly shaped masses that distorted and sometimes partially compressed surrounding tissue (Plate 1). They were composed of poorly defined, glandular and/or papillary like structures lined by one to a few rows of loosely to densely packed, generally uniform, well-differentiated cuboidal epithelial cells (Plate 2). In some instances, adenomas were located within large areas of marked, noncompressive, alveolar epithelial hyperplasia; however adenoma cells were usually larger than those in areas of alveolar epithelial hyperplasia. Alveolar/bronchiolar carcinomas were generally larger, irregularly shaped, expansile and invasive masses that effaced the normal alveolar architecture and compressed the surrounding parenchyma (Plate 3). Component neoplastic cells were densely packed, pleomorphic and had a heterogeneous growth with areas of poorly defined, irregular, glandular and/or papillary structures and occasionally solid sheets, incompletely separated by fibrous trabeculae (Plate 4). The cells had oval to round, hyperchromatic to vesicular nuclei often with prominent nucleoli and variable amounts of often finely vacuolated cytoplasm. Carcinomas contained scattered foci of necrosis and/or neutrophils.

The cystic keratinizing epithelioma had the characteristic morphology of this neoplasm type. It was a large, compressive mass with an irregular, thick wall of multilayered, well-differentiated squamous epithelium that surrounded a large central cavity packed with massive accumulations of lamellated to whorled keratin aggregates, necrotic debris, and inflammatory cells (Plates 5 and 6). The squamous-type lining wall of the cystic keratinizing epithelioma was in close contact and focally contiguous with an adjacent area of alveolar epithelial squamous metaplasia. The toxicological significance of the neoplasm in this study is unknown due to the single occurance in one female rat.

Exposure to TRIM VX resulted in increased incidences of a complex spectrum of nonneoplastic lesions in the lung. The lesions were multifocal to locally extensive and were frequently intermingled and contiguous such that it was sometimes difficult to establish clear demarcations between the various changes. There were significantly increased incidences of alveolar epithelium hyperplasia, alveolar/bronchiolar epithelium hyperplasia, fibrosis, histiocytic cellular infiltration, and chronic active inflammation in all exposed groups of males and females (Tables 9, A4, and B4). There were significantly increased incidences of alveolar epithelium squamous metaplasia and lymphohistiocytic hyperplasia of bronchus-associated lymphoid tissue (BALT) in 100 mg/m^3 males and 30 and 100 mg/m^3 females. There were also significantly increased incidences of alveolus proteinosis in 30 and 100 mg/m^3 males and all exposed groups of females. The severity of these lesions generally increased with exposure concentration.

Alveolar epithelium hyperplasia consisted of multifocal to confluent, discrete, irregularly shaped, minimal to marked proliferations of alveolar epithelial cells in which the alveolar architecture was still discernable (Plate 7). Component epithelial cells that lined the alveolar septa were well-differentiated, plump, and typically cuboidal cells (Plate 8). There was minimal focal crowding and disorganization. The hyperplastic areas blended inconspicuously with adjacent normal alveolar parenchyma and ranged from small foci composed of only a few alveoli to extensive zones that could involve most of an individual lobe, but even the largest hyperplasias did not cause compression of the adjacent parenchyma. Areas of alveolar epithelial hyperplasia were often intermingled or contiguous with areas of other treatment-related nonneoplastic lung lesions.

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Respiratory System in Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Male				
Lung[a]	50	50	50	50
Alveolar Epithelium, Hyperplasia[b]	11 (1.2)[c]	43** (1.3)	45** (1.5)	49** (2.1)
Alveolar Epithelium, Metaplasia, Squamous	0	0	0	5* (1.4)
Alveolar/bronchiolar Epithelium, Hyperplasia	4 (1.0)	22** (1.0)	39** (1.2)	46** (1.8)
Alveolus, Proteinosis	0	1 (1.0)	31** (1.2)	45** (2.2)
Bronchus-associated Lymphoid Tissue, Hyperplasia, Lymphohistiocytic	0	1 (1.0)	4 (1.3)	6* (1.0)
Fibrosis	4 (1.0)	43** (1.0)	45** (1.4)	49** (1.3)
Infiltration Cellular, Histiocyte	14 (1.0)	50** (1.4)	50** (2.0)	50** (2.6)
Inflammation, Chronic Active	7 (1.0)	46** (1.0)	46** (2.0)	48** (3.0)
Alveolar/bronchiolar Adenoma[d]	0	0	0	1
Alveolar/bronchiolar Carcinoma[e]				
Overall rate[f]	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adjusted rate[g]	0.0%	0.0%	0.0%	4.8%
Terminal rate[h]	0/36 (0%)	0/39 (0%)	0/33 (0%)	2/34 (6%)
First incidence (days)	—[j]	—	—	729 (T)
Poly-3 test[i]	P=0.036	—[k]	—	P=0.221
Alveolar/bronchiolar Adenoma or Carcinoma[d]				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	7.2%
Terminal rate	0/36 (0%)	0/39 (0%)	0/33 (0%)	3/34 (9%)
First incidence (days)	—	—	—	729 (T)
Poly-3 test	P=0.007	—	—	P=0.106
Nose	50	50	50	50
Glands, Olfactory Epithelium, Hyperplasia	0	43** (1.5)	41** (1.6)	49** (1.9)
Goblet Cell, Hyperplasia	5 (1.4)	36** (1.0)	37** (1.1)	42** (1.2)
Inflammation, Suppurative	7 (1.1)	46** (1.2)	47** (1.3)	46** (1.1)
Olfactory Epithelium, Accumulation, Hyaline Droplet	20 (1.4)	50** (2.8)	50** (2.9)	50** (3.2)
Respiratory Epithelium, Accumulation, Hyaline Droplet	10 (1.2)	50** (1.4)	48** (1.4)	50** (1.6)
Respiratory Epithelium, Hyperplasia	2 (1.0)	7 (1.0)	7 (1.0)	19** (1.1)
Transitional Epithelium, Hyperplasia	7 (1.3)	7 (1.0)	8 (1.4)	13 (1.5)
Larynx	50	50	50	50
Epiglottis, Hyperplasia, Squamous	0	26** (1.1)	48** (1.8)	50** (2.4)
Epiglottis, Metaplasia, Squamous	3 (1.0)	50** (1.4)	50** (2.1)	50** (2.6)
Infiltration Cellular, Mixed Cell	1 (1.0)	9* (1.1)	27** (1.0)	31** (1.1)
Lymph Node, Bronchial	39	43	42	42
Hyperplasia, Lymphohistiocytic	0	17** (1.4)	29** (1.8)	35** (1.9)
Lymph Node, Mediastinal	43	48	44	43
Hyperplasia, Lymphohistiocytic	0	20** (1.5)	22** (1.9)	32** (2.5)

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Respiratory System in Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m³	30 mg/m³	100 mg/m³
Female				
Lung	50	50	50	50
Alveolar Epithelium, Hyperplasia	8 (1.3)	43** (1.1)	49** (2.0)	50** (2.4)
Alveolar Epithelium, Metaplasia, Squamous	0	3 (1.3)	9** (1.3)	21** (1.9)
Alveolar/bronchiolar Epithelium, Hyperplasia	2 (1.0)	9* (1.1)	31** (1.4)	50** (2.8)
Alveolus, Proteinosis	1 (1.0)	15** (1.0)	41** (1.4)	48** (2.8)
Bronchus-associated Lymphoid Tissue, Hyperplasia, Lymphohistiocytic	0	2 (1.0)	7** (1.0)	10** (1.4)
Fibrosis	5 (1.0)	35** (1.1)	49** (1.5)	50** (1.8)
Infiltration Cellular, Histiocyte	16 (1.1)	48** (1.3)	50** (1.8)	50** (2.8)
Inflammation, Chronic Active	5 (1.0)	46** (1.0)	50** (2.0)	50** (3.2)
Cystic Keratinizing Epithelioma[l]	0	0	0	1
Alveolar/bronchiolar Adenoma, Multiple	0	0	0	2
Alveolar/bronchiolar Adenoma (includes multiple)[l]				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	2.3%	6.8%
Terminal rate	0/30 (0%)	0/33 (0%)	1/33 (3%)	3/30 (10%)
First incidence (days)	—	—	731 (T)	731 (T)
Poly-3 test	P=0.024	—	P=0.511	P=0.127
Nose	50	50	49	50
Glands, Olfactory Epithelium, Hyperplasia	0	32** (1.1)	43** (1.1)	46** (1.5)
Goblet Cell, Hyperplasia	3 (1.0)	36** (1.0)	40** (1.1)	47** (1.1)
Inflammation, Suppurative	1 (1.0)	46** (1.2)	47** (1.2)	48** (1.3)
Olfactory Epithelium, Accumulation, Hyaline Droplet	14 (1.2)	50** (2.8)	49** (2.9)	50** (3.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	5 (1.2)	50** (1.5)	48** (1.5)	50** (1.5)
Respiratory Epithelium, Hyperplasia	0	7** (1.0)	8** (1.0)	25** (1.0)
Transitional Epithelium, Hyperplasia	5 (1.2)	11 (1.1)	4 (1.0)	21** (1.3)
Larynx	50	50	50	50
Epiglottis, Hyperplasia, Squamous	1 (1.0)	24** (1.6)	41** (1.4)	50** (2.3)
Epiglottis, Metaplasia, Squamous	0	49** (1.4)	50** (2.2)	50** (2.7)
Infiltration Cellular, Mixed Cell	0	9** (1.0)	18** (1.1)	22** (1.1)
Lymph Node, Bronchial	37	37	44	36
Hyperplasia, Lymphohistiocytic	1 (1.0)	18** (1.3)	37** (1.6)	31** (1.8)
Lymph Node, Mediastinal	46	49	46	45
Hyperplasia, Lymphohistiocytic	0	11** (1.3)	14** (1.6)	28** (2.2)

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Respiratory System in Rats in the 2-Year Inhalation Study of TRIM VX

* Significantly different (P≤0.05) from the chamber control group by the Poly-3 test
** P≤0.01
(T) Terminal kill
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 4/150 (2.7% ± 3.1%), range 0%-6%; all routes: 4/299 (1.3% ± 2.4%), range 0%-6%
[e] Historical incidence for inhalation studies: 0/150; all routes: 0/299
[f] Number of animals with neoplasm per number of animals with lung examined microscopically
[g] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[h] Observed incidence at terminal kill
[i] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[j] Not applicable; no neoplasm in animal group
[k] Value of statistic cannot be computed.
[l] Historical incidence for inhalation studies: 0/150; all routes: 0/300

Alveolar/bronchiolar epithelium hyperplasia consisted of multifocal cellular proliferations in which the distal terminal bronchioles, alveolar ducts, and immediately adjacent septa (periacinar region) were lined by single to few crowded layers of tall cuboidal to columnar ciliated cells that had vesicular oval nuclei and pale eosinophilic cytoplasm (Plates 9 and 10). These changes did not extend into the more proximal terminal bronchioles. Alveolar epithelium squamous metaplasia consisted of scattered, small to quite large, irregular foci in which the alveolar septa and sometimes the alveolar ducts were lined by multiple layers of well-differentiated squamous epithelium (Plates 11 and 12). Superficial keratinization was generally present and in some areas was so abundant as to occlude affected alveoli. Areas of alveolar epithelium hyperplasia, alveolar/bronchiolar epithelium hyperplasia, and alveolar squamous metaplasia were often contiguous and/or admixed with each other, often without clear demarcation between these changes.

Infiltration, cellular, histiocyte consisted of multifocal variably sized areas in which the alveoli contained few to many macrophages (histiocytes). The infiltrates were mixtures of small to swollen macrophages that typically had small, round to oval nuclei and abundant, pale pink to grayish, finely granular or foamy cytoplasm (Plates 13 and 14). Frequently, the cells were distended with deeply eosinophilic proteinaceous material that sometimes contained angular eosinophilic crystals. Histiocytic cell infiltrates were often closely intermingled with intra-alveolar eosinophilic protein material (proteinosis), but frequently, histiocytes also occurred in alveoli without this material. The infiltrates were often confluent with other lung lesions, especially inflammation, such that it was sometimes difficult to distinguish demarcations between the various nonneoplastic lesions. Alveolus proteinosis consisted of predominantly amorphous, pale, flocculent, wispy, light pink to deeply eosinophilic proteinaceous material primarily within the alveoli and sometimes within the alveolar ducts (Plates 15 and 16). Occasional scattered clumps were dense and deeply eosinophilic. Severity ranged from minimal to marked, with higher grade lesions exhibiting copious amounts of intra-alveolar protein which filled many or most alveoli in any given lung lobe. Less extensive lesions were characterized by scattered areas of affected alveoli containing variable amounts of proteinaceous material. Frequently, the material contained deeply eosinophilic angular crystals. This material was often but not always associated with intra-alveolar histiocyte infiltrates, areas of alveolar epithelium hyperplasia, and chronic active inflammation.

Chronic active inflammation was a complex lesion that consisted of multifocal to focally extensive areas of mild to markedly intense aggregations of inflammatory cells that obscured and/or effaced the alveolar parenchyma (Plate 17). Although areas of inflammation were scattered throughout the pulmonary parenchyma, they were frequently located peripherally, adjacent to the pleural surface. The inflammatory cell infiltrates consisted primarily of macrophages, epithelioid cells, and occasional multinucleated giant cells, along with variable numbers of lymphocytes, small monocytes, and red blood cells (Plate 18). Some epithelioid macrophages and less often multinucleated giant cells contained clear, spindle-shaped clefts, resembling sterol clefts. Occasionally, few areas of inflammation consisted primarily of neutrophils or lymphocytes mixed with lesser numbers of macrophages. Variable fibrosis was consistently present within areas of inflammation.

Fibrosis of the lung was characterized by the presence of fibrous collagenous strands within the alveolar interstitium that resulted in subtle, though widespread, thickening of alveolar septa and alveolar duct walls including areas of alveolar epithelial hyperplasia (Plates 17 and 18). Variable fibrous strands and bands also often extended into alveolar spaces, variably within and surrounding areas of chronic active inflammation. Fibrous sheaths were also often especially prominent around affected alveoli/alveolar ducts in areas of alveolar epithelium squamous metaplasia, especially the larger areas. Frequently, there was fibrosis of the parietal pleura characterized by focal to confluent, subtle to prominent thickening of the pleura due to increased amounts of mature collagenous tissue. In some cases, multiple, delicate fibrous tags protruded from the thickened pleural surface; these tags were covered by single layers of well-differentiated mesothelial cells. In some areas, low numbers of lymphocytes, macrophages, and/or mast cells were scattered though the fibrotic pleura. Oil-red-O histochemical staining of lung sections revealed oil droplets (presumed to be a component of TRIM VX) free within the inflammatory lesions and within alveolar macrophages and the hyperplastic alveolar epithelium (Plate 19).

Lymphohistiocytic hyperplasia of BALT was variably enlarged due to increased numbers of well-differentiated lymphocytes mixed with multifocal to coalescing clusters of large macrophages that had abundant, foamy, pale amphophilic cytoplasm (Plates 20 and 21).

Nose: There were significantly increased incidences of olfactory epithelium glands hyperplasia, goblet cell hyperplasia, suppurative inflammation, and olfactory and respiratory epithelium hyaline droplet accumulation in all exposed groups of males and females (Tables 9, A4, and B4). There were also significantly increased incidences of respiratory epithelium hyperplasia in 100 mg/m^3 males and all exposed groups of females and transitional epithelium hyperplasia in 100 mg/m^3 females.

Suppurative inflammation was a minimal to mild change that was most common in the ventral ethmoid turbinates of the olfactory epithelium. This lesion was characterized by infiltrates of predominantly neutrophils mixed with fewer mononuclear cells within the lamina propria and sometimes the lumens of hyperplastic olfactory epithelial glands (Plate 22). In some cases, focal neutrophil infiltrates were also noted in the lamina propria and/or epithelium of the nasoturbinates, maxilloturbinates, and/or the lateral walls in the Level I and II nasal sections.

Respiratory epithelium hyperplasia of minimal severity was a focal lesion generally confined to the distal tips of the Level II maxilloturbinates and less commonly the tips of the nasoturbinates. Hyperplasia consisted of focally increased numbers of epithelial cells lining the affected turbinates resulting in small segments of densely packed, tall columnar epithelial cells with elongated, hyperchromatic nuclei (Plate 23).

Transitional epithelium hyperplasia of minimal to mild severity was located along the tips and lateral surfaces and curvatures of the nasoturbinates and maxilloturbinates and/or the adjacent lateral meatus walls in the Level I section of the nose. Hyperplasia appeared as focal to segmental areas of hypercellularity of the epithelium due to increased numbers (proliferation) of the epithelial cells (Plate 24).

Respiratory epithelium hyaline droplet accumulation of minimal to mild severity most commonly affected the epithelium at the bases of the ventral ethmoid turbinates and along the ventral meatus floor. Affected cells were distended by large aggregates of intracytoplasmic, supranuclear, homogeneous, brightly eosinophilic globular material that partially or completely filled the cytoplasm. The affected segments of epithelium appeared folded due to crowding of the swollen epithelial cells. Olfactory epithelium hyaline droplet accumulation of mild to moderate severity was morphologically similar to that of the respiratory epithelium and involved the olfactory epithelium lining the ventral ethmoid turbinates and underlying submucosal glands (Plate 25).

Olfactory epithelium glands hyperplasia occurred in the submucosal glands in the ethmoid region of the nose especially in the more ventral ethmoid turbinates and ventral meatus. Affected glands were increased in size and there appeared to be increased numbers of gland profiles (Plate 26).

Goblet cell hyperplasia of mostly minimal severity most commonly affected the respiratory epithelium lining the mid to distal nasoturbinates and maxilloturbinates in Level II. In affected segments of the epithelium, the numbers and/or size of the mucus-containing goblet cells that are normal components of respiratory epithelium were increased. Hyperplasia was sporadic and less prominent in other areas of the respiratory epithelium regions and along the nasal septum and lateral walls in Levels I and II.

Larynx: There were significantly increased incidences of epiglottis squamous hyperplasia, epiglottis squamous metaplasia, and mixed cell infiltration cellular in all exposed male and female groups (Tables 9, A4, and B4). Squamous metaplasia and squamous hyperplasia of the epiglottis were minimal to moderate changes that affected the epithelium in the histologic section that contains the epiglottis. Squamous metaplasia was characterized by replacement of the cuboidal to low columnar, ciliated epithelium that normally lines the base and lateral aspects of the epiglottis by multiple layers of well-differentiated, variably keratinized squamous epithelium (Plate 27). In more severe cases, the metaplastic change affected the entire epithelium lining base of the epiglottis and lower aspects of the lateral walls, and the central portion of the base in less severe cases. Squamous hyperplasia was characterized by thickening of the squamous epithelium that normally lines the arytenoid cartilages (Plate 27).

Bronchial and Mediastinal Lymph Nodes: There were significantly increased incidences of lymphohistiocytic hyperplasia in all exposed groups of males and females (Tables 9, A4, and B4) and the severities increased with exposure concentration. The morphology of this lesion was similar to that observed in the lung. The lymph nodes were large due to expansion of the cortex and paracortex by increased numbers of well-differentiated small lymphocytes mixed with focal, multifocal to coalescing aggregates of plump macrophages with abundant, foamy, pale amphophilic cytoplasm (Plate 28). The macrophage aggregates were morphologically distinct from the pigment-laden (brown) macrophages normally present in the medullary cords and sinusoids of the chamber.

MICE

3-MONTH STUDY

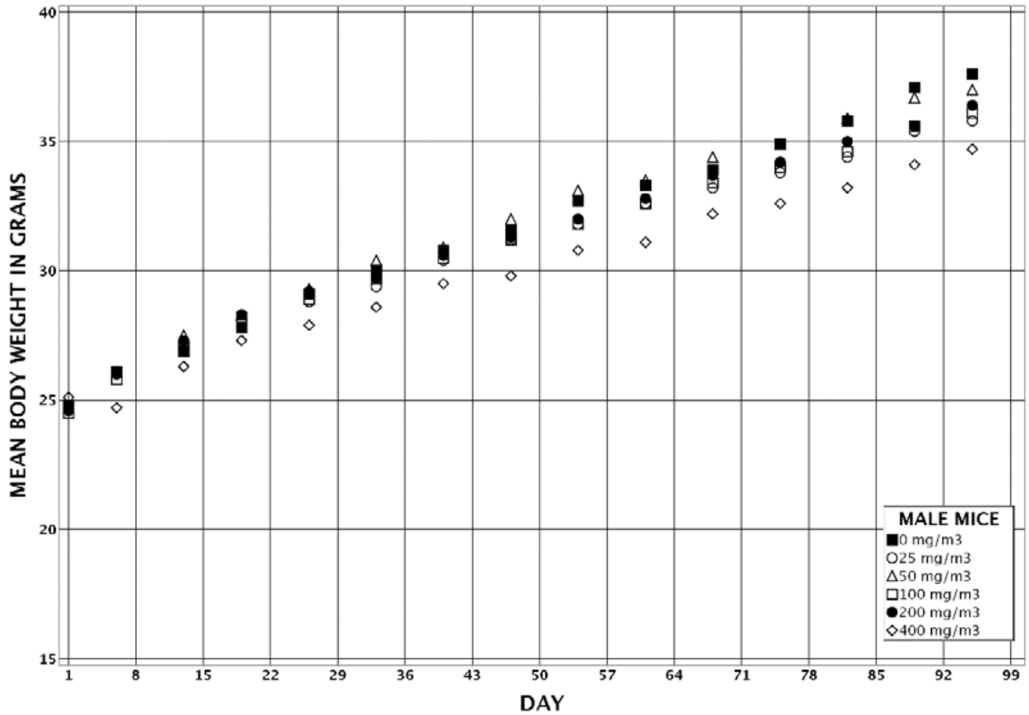
All mice survived to the end of the study and the mean body weights of exposed groups of females were similar to those of the chamber controls (Table 10; Figure 4). Final mean body weights and mean body weight gains of 400 mg/m^3 males were significantly less than those of the chamber controls. There were no chemical-related clinical observations in male or female mice (NTP, 2016c).

Mild decreases (≤12%) in the erythrocyte and reticulocyte counts and packed cell volume occurred in 200 and 400 mg/m^3 males (Table F2). Hemoglobin concentration was also mildly decreased in 200 mg/m^3 males. The exact etiology for the decreases in the erythron is not known; however, the possibility exists that the mild suppression is due to the systemic effects of the chronic inflammation present in the respiratory tract. There were no changes in the hematology parameters of female mice.

TABLE 10
Survival and Body Weights of Mice in the 3-Month Inhalation Study of TRIM VX[a]

Concentration (mg/m^3)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	24.8 ± 0.3	37.6 ± 0.8	12.8 ± 0.7	
25	10/10	24.7 ± 0.3	35.8 ± 0.4	11.1 ± 0.3	95
50	10/10	24.7 ± 0.3	37.0 ± 0.7	12.3 ± 0.7	98
100	10/10	24.5 ± 0.3	36.1 ± 0.8	11.6 ± 0.7	96
200	10/10	24.6 ± 0.4	36.4 ± 0.6	11.8 ± 0.4	97
400	10/10	25.1 ± 0.3	34.7 ± 0.6*	9.7 ± 0.5**	92
Female					
0	10/10	19.9 ± 0.3	29.8 ± 0.9	9.9 ± 0.8	
25	10/10	20.2 ± 0.3	31.0 ± 0.9	10.8 ± 0.7	104
50	10/10	19.5 ± 0.3	30.7 ± 1.1	11.3 ± 0.9	103
100	10/10	19.7 ± 0.3	29.1 ± 0.8	9.4 ± 0.8	98
200	10/10	19.8 ± 0.2	30.1 ± 0.6	10.3 ± 0.5	101
400	10/10	20.1 ± 0.2	29.2 ± 0.5	9.2 ± 0.6	98

* Significantly different (P≤0.05) from the chamber control group by Dunnett's test
** Significantly different (P≤0.01) from the chamber control group by William's test
[a] Weights and weight changes are given as mean ± standard error.
[b] Number of animals surviving at 14 weeks/number initially in group



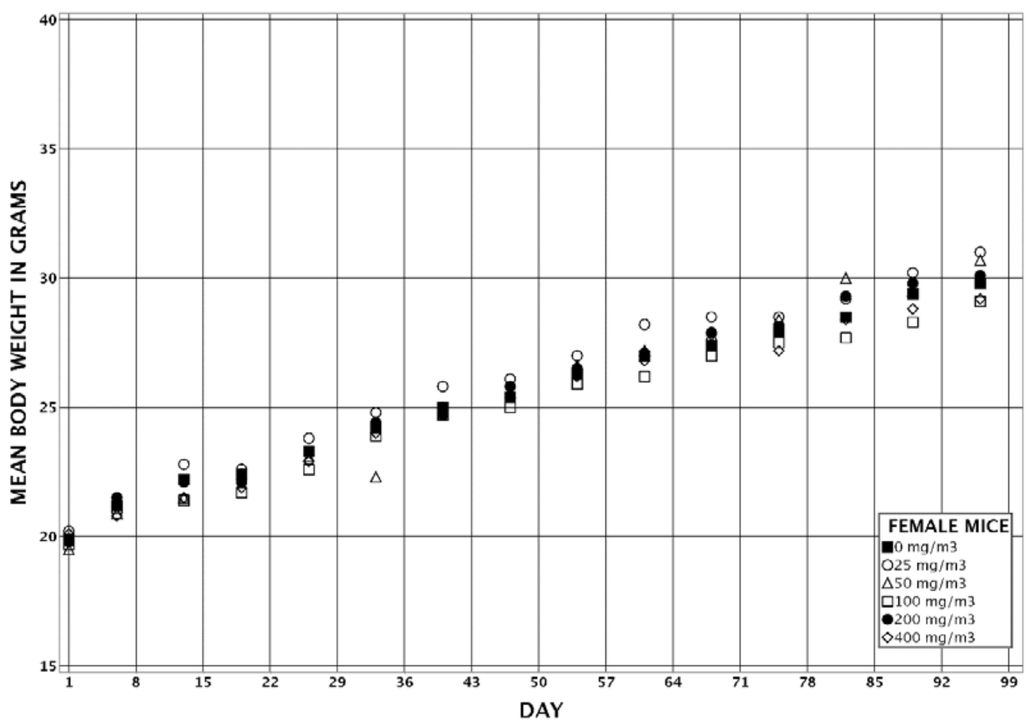


FIGURE 4
Growth Curves for Mice Exposed to TRIM VX by Inhalation for 3 Months

The absolute liver weights of 200 and 400 mg/m^3 males and females and the relative liver weights of all exposed groups of males and 200 and 400 mg/m^3 females were significantly greater (up to 28%) than those of the chamber control groups (Tables 11 and G2); there were no corresponding histopathologic lesions in the liver. The absolute lung weights of 200 and 400 mg/m^3 males and the relative lung weights of 100, 200, and 400 mg/m^3 males were significantly increased (up to 44%). In females, the absolute and relative lung weights of the 100, 200, and 400 mg/m^3 groups were significantly increased (up to 49%). The absolute and relative spleen weights of males exposed to 50 mg/m^3 or greater and the relative spleen weight of 400 mg/m^3 females were significantly increased (up to 31%). There were no corresponding histopathologic lesions in the spleen.

In the lung, the incidences of fibrosis and infiltration cellular histiocytic in males and females exposed to 100 mg/m^3 or greater were significantly greater than those of the chamber control groups (Table 12). There were significantly increased incidences of chronic active inflammation in males exposed to 50 mg/m^3 or greater and females exposed to 100 mg/m^3 or greater. There were also significantly increased incidences of bronchiole hyperplasia in males and females exposed to 50 mg/m^3 or greater. Fibrosis was characterized by increased deposition of collagen in the interstitial tissue at the junction of the terminal bronchioles and alveolar ducts and extending into alveolar ducts and occasionally adjacent alveoli. Masson's trichrome staining of mouse lung confirmed the presence of increased interstitial collagen. The severity of inflammation chronic active was

TABLE 11
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Inhalation Study of TRIM VX[a]

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
n	10	10	10	10	10	10
Male						
Necropsy body wt	37.6 ± 0.8	35.8 ± 0.4	37.0 ± 0.7	36.1 ± 0.8	36.4 ± 0.6	34.7 ± 0.6*
Liver						
Absolute	1.54 ± 0.05	1.58 ± 0.03	1.71 ± 0.05	1.64 ± 0.04	1.81 ± 0.07**	1.76 ± 0.05**
Relative	40.795 ± 0.675	43.982 ± 0.513*	46.136 ± 1.159**	45.386 ± 0.861**	49.719 ± 1.359**	50.636 ± 0.900**
Lung						
Absolute	0.23 ± 0.02	0.21 ± 0.00	0.23 ± 0.01	0.25 ± 0.01	0.29 ± 0.01**	0.30 ± 0.00**
Relative	6.044 ± 0.420	5.862 ± 0.113	6.188 ± 0.189	6.886 ± 0.167*	7.581 ± 0.162**	8.684 ± 0.135**
Spleen						
Absolute	0.061 ± 0.002	0.065 ± 0.002	0.072 ± 0.003**	0.070 ± 0.003*	0.077 ± 0.004**	0.068 ± 0.002**
Relative	1.621 ± 0.046	1.815 ± 0.049	1.948 ± 0.070**	1.949 ± 0.085**	2.120 ± 0.106**	1.961 ± 0.057**
Female						
Necropsy body wt	29.8 ± 0.9	31.0 ± 0.9	30.7 ± 1.1	29.1 ± 0.8	30.1 ± 0.6	29.2 ± 0.5
Liver						
Absolute	1.35 ± 0.04	1.50 ± 0.06	1.51 ± 0.06	1.37 ± 0.04	1.57 ± 0.02**	1.70 ± 0.04**
Relative	45.360 ± 1.019	48.331 ± 0.916	49.196 ± 1.141	46.971 ± 1.036	52.298 ± 1.057**	58.078 ± 1.027**
Lung						
Absolute	0.21 ± 0.00	0.22 ± 0.01	0.22 ± 0.01	0.25 ± 0.01**	0.27 ± 0.01**	0.30 ± 0.01**
Relative	6.973 ± 0.262	7.103 ± 0.122	7.284 ± 0.255	8.519 ± 0.276**	9.123 ± 0.256**	10.382 ± 0.181**
Spleen						
Absolute	0.093 ± 0.003	0.096 ± 0.005	0.097 ± 0.004	0.089 ± 0.003	0.102 ± 0.003	0.106 ± 0.003
Relative	3.129 ± 0.088	3.090 ± 0.102	3.167 ± 0.113	3.057 ± 0.077	3.391 ± 0.095	3.634 ± 0.098**

* Significantly different (P≤0.05) from the chamber control group by Williams' or Dunnett's test
** (P≤0.01)
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE 12
Incidences of Nonneoplastic Lesions of the Respiratory System in Mice in the 3-Month Inhalation Study of TRIM VX

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
Male						
Lung[a]	10	10	10	10	10	10
Fibrosis[b]	0	0	1 (1.0)[c]	8** (1.0)	10** (1.1)	10** (3.0)
Infiltration Cellular, Histiocyte	0	0	2 (1.0)	9** (1.2)	10** (1.2)	10** (1.5)
Inflammation, Chronic Active	0	0	4* (1.0)	10** (1.2)	10** (2.0)	10** (3.0)
Bronchiole, Hyperplasia	0	0	9** (1.0)	10** (1.1)	10** (2.1)	10** (2.8)
Nose	10	10	10	10	10	10
Inflammation, Suppurative	0	7** (1.0)	8** (1.0)	8** (1.0)	8** (1.0)	10** (1.0)
Olfactory Epithelium, Accumulation, Hyaline Droplet	0	10** (1.3)	10** (1.6)	10** (2.0)	10** (2.0)	10** (2.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	0	10** (1.1)	10** (1.0)	10** (1.0)	10** (1.4)	10** (1.3)
Larynx	10	10	10	10	10	10
Hyperplasia, Squamous	0	0	2 (1.5)	9** (1.7)	8** (1.5)	9** (1.9)
Inflammation, Chronic	0	4* (1.0)	0	3 (1.0)	1 (1.0)	0
Metaplasia, Squamous	0	10** (2.2)	10** (4.0)	10** (4.0)	10** (4.0)	10** (4.0)
Female						
Lung	10	10	10	10	10	10
Fibrosis	0	0	0	10** (1.0)	10** (1.7)	10** (2.9)
Infiltration Cellular, Histiocyte	0	0	1 (1.0)	5* (1.4)	9** (1.1)	10** (1.8)
Inflammation, Chronic Active	1 (1.0)	0	2 (1.0)	10** (1.4)	10** (2.2)	10** (3.0)
Bronchiole, Hyperplasia	0	0	4* (1.0)	10** (1.4)	10** (2.0)	10** (2.9)
Nose	10	10	10	10	10	10
Inflammation, Suppurative	0	1 (1.0)	6** (1.0)	5* (1.0)	10** (1.0)	7** (1.0)
Olfactory Epithelium, Accumulation, Hyaline Droplet	0	10** (1.5)	10** (2.0)	10** (2.0)	10** (2.0)	10** (2.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	0	10** (1.3)	10** (1.7)	10** (2.1)	10** (2.1)	10** (2.1)
Larynx	10	10	10	10	10	10
Hyperplasia, Squamous	0	0	1 (2.0)	7** (1.9)	7** (2.0)	8** (2.0)
Inflammation, Chronic	0	4* (1.0)	5* (1.0)	8** (1.0)	9** (1.0)	5* (1.0)
Metaplasia, Squamous	0	10** (2.2)	10** (2.6)	10** (3.4)	10** (4.0)	10** (4.0)

* Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact test
** $P \leq 0.01$
a Number of animals with tissue examined microscopically
b Number of animals with lesion
c Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

minimal to moderate in the lungs of all mice exposed to 100 mg/m^3 or more and minimal in a few mice exposed to 50 mg/m^3, and was characterized by increased numbers of alveolar macrophages and occasional neutrophils in alveolar spaces. There also were mononuclear cell infiltrates in alveolar septa and walls of alveolar ducts. Infiltration cellular histiocytic was characterized by the presence of large, vacuolated alveolar macrophages which were scattered throughout the alveolar spaces. Bronchiole hyperplasia, ranging from minimal to moderate in severity, was characterized by increased cellularity in the terminal bronchioles with apparent epithelial proliferation and piling up at the junctions of the terminal bronchioles and alveolar ducts.

In the nose, there were significantly increased incidences of hyaline droplet accumulation of the olfactory and respiratory epithelium in all exposed groups of males and females (Table 12). There were also significantly increased incidences of suppurative inflammation in all exposed groups of males and in females exposed to 50 mg/m^3 or greater. Hyaline droplet accumulation was minimal to mild in the olfactory epithelium of nasal Level III in all exposed mice. It was most prominent on the ventro-lateral regions of the ethmoturbinates. Accumulation of hyaline droplets in the respiratory epithelium was minimal to moderate in nearly all exposed mice, and involved the nasopharyngeal duct in Level III and the ventral nasal septum and/or lateral walls in Levels I and/or II. Suppurative inflammation consisted of small numbers of neutrophils scattered throughout the ethmoturbinate submucosa subjacent to the areas of hyaline droplet accumulation.

In the larynx, there were significantly increased incidences of squamous metaplasia in all exposed groups of males and females, squamous hyperplasia in males and females exposed to 100 mg/m^3 or greater, and chronic inflammation in all exposed groups of females (Table 12). Squamous metaplasia involved the ventral epithelium at the base of the epiglottis, lateral walls of the anterior section (Level I), and dorsolateral walls and ventral diverticulum of the middle section (Level II). The normally pseudostratified ciliated columnar epithelium lining the base of the epiglottis and nonciliated cuboidal epithelium lining the ventral diverticulum were replaced by mature, sometimes keratinized, squamous epithelium. The metaplastic squamous epithelium was frequently thickened thus appearing hyperplastic as well. Squamous hyperplasia was characterized by thickening of the epithelium (more than three cell layers versus one to two cell layers in chamber controls) overlying the arytenoid cartilages in the middle section (Level II). Chronic inflammation in exposed mice consisted of submucosal infiltrates of small numbers of lymphocytes and plasma cells in anterior (Level I) and middle (Level II) laryngeal sections.

Exposure Concentration Selection Rationale: The exposure concentrations selected for the 2-year inhalation study in male and female B6C3F1/N mice were 10, 30, and 100 mg/m^3. The highest exposure concentration was based on the incidence and severity of lung fibrosis in the current 3-month study. These concentrations were also used in the 2-year study of CIMSTAR 3800 in B6C3F1/N mice which will allow for comparisons between the two metalworking fluid studies.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 13 and in the Kaplan-Meier survival curves (Figure 5). Survival of all exposed groups of male and female mice was similar to that of the chamber control groups.

Body Weights and Clinical Findings

The mean body weights of all exposed groups of males and females were similar to those of the chamber control groups throughout the study (Tables 14 and 15; Figure 6). Clinical observations included abnormal breathing in a few exposed males and females but the occurrences did not appear to be dose related (NTP, 2016d).

TABLE 13
Survival of Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Male				
Animals initially in study	50	50	50	50
Accidental death[a]	0	0	1	0
Moribund	4	9	6	5
Natural deaths	8	2	6	8
Animals surviving to study termination	38	39	37[e]	37
Percent probability of survival at end of study[b]	76	78	76	74
Mean survival (days)[c]	692	701	688	701
Survival analysis[d]	P=0.877	P=0.937N	P=1.000	P=1.000
Female				
Animals initially in study	50	50	50	50
Accidental death	1	0	0	0
Moribund	10	9	4	13
Natural deaths	4	5	10	7
Animals surviving to study termination	35[e]	36	36	30[e]
Percent probability of survival at end of study	71	72	72	60
Mean survival (days)	692	697	700	684
Survival analysis	P=0.196	P=1.000	P=0.997N	P=0.370

a Censored in the survival analyses
b Kaplan-Meier determinations
c Mean of all deaths (uncensored, censored, and terminal kill)
d The result of the life table trend test (Tarone, 1975) is in the chamber control column, and the results of the life table pairwise comparisons (Cox, 1972) with the chamber controls are in the exposed group columns. A lower mortality in an exposure group is indicated by **N**.
e Includes one animal that died during the last week of the study

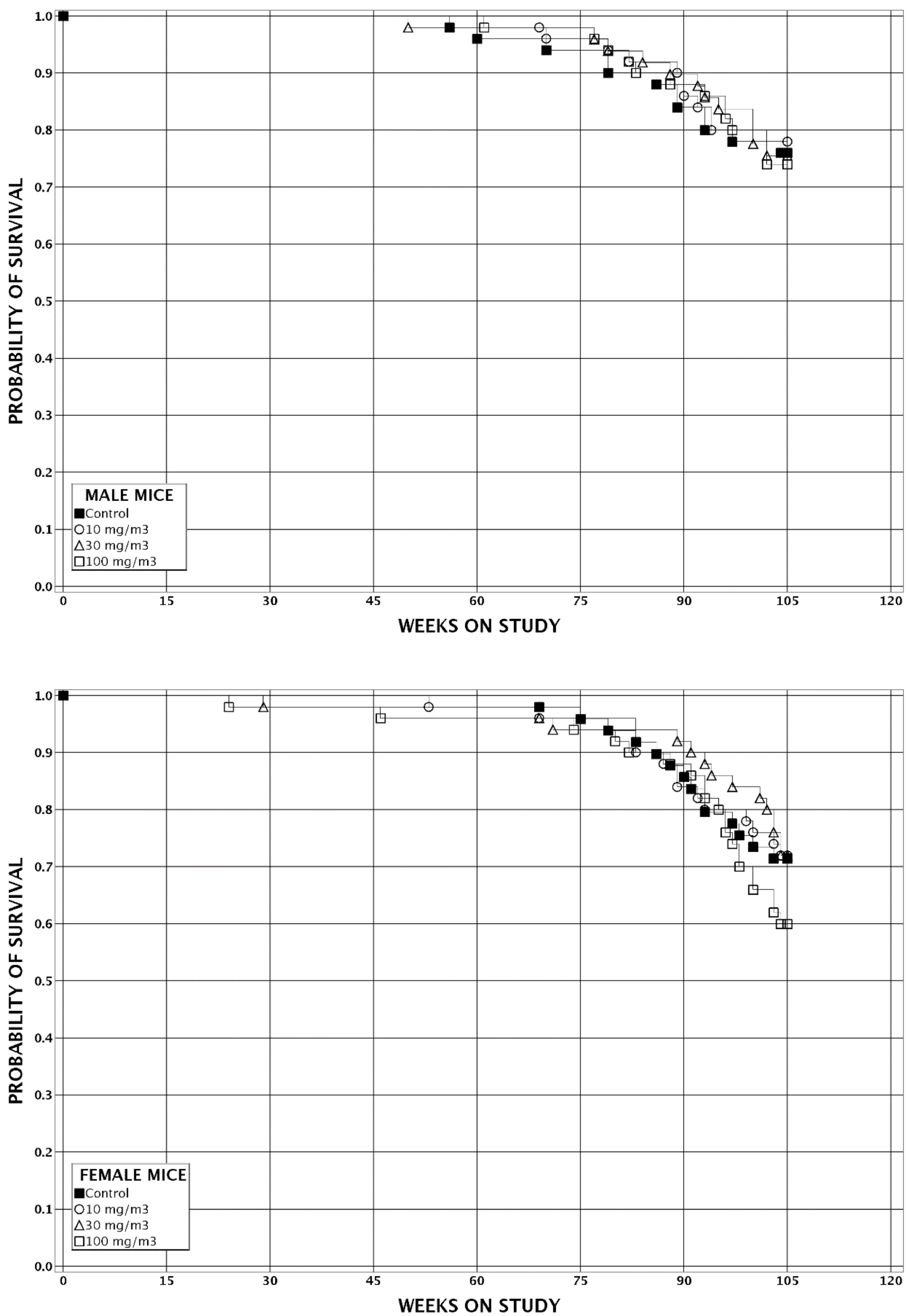


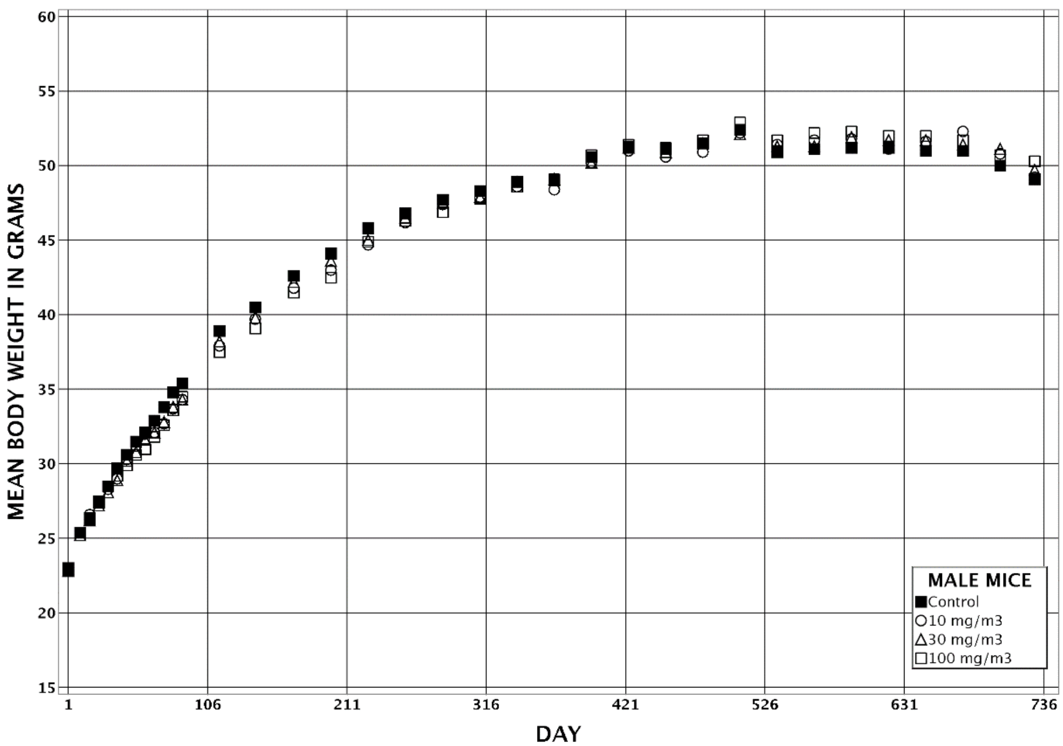
FIGURE 5
Kaplan-Meier Survival Curves for Mice Exposed to TRIM VX by Inhalation for 2 Years

TABLE 14
Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control		10 mg/m^3			30 mg/m^3			100 mg/m^3		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	23.0	50	22.9	100	50	22.8	99	50	22.9	100	50
10	25.4	50	25.4	100	50	25.2	99	50	25.2	100	50
17	26.4	50	26.6	101	50	26.2	99	50	26.3	100	50
24	27.5	50	27.4	100	50	27.2	99	50	27.4	100	50
31	28.5	50	28.3	99	50	28.1	99	50	28.5	100	50
38	29.7	50	29.0	98	50	28.9	98	50	29.2	99	50
45	30.6	50	30.3	99	50	30.2	99	50	29.9	98	50
52	31.5	50	31.2	99	50	30.8	98	50	30.6	97	50
59	32.1	50	32.0	100	50	31.6	99	50	31.0	97	50
66	32.9	50	32.1	97	50	32.1	98	50	31.8	97	50
73	33.8	50	32.7	97	50	32.8	97	49	32.6	97	50
80	34.8	50	33.7	97	50	33.8	97	49	33.6	97	50
87	35.4	50	34.3	97	50	34.3	97	49	34.5	97	50
115	38.9	50	37.9	97	50	38.2	98	49	37.5	96	50
142	40.5	50	39.7	98	50	39.8	98	49	39.1	97	50
171	42.6	50	41.8	98	50	42.2	99	49	41.5	97	50
199	44.1	50	43.0	97	50	43.6	99	49	42.5	96	50
227	45.8	50	44.7	98	50	45.0	98	49	44.9	98	50
255	46.8	50	46.2	99	50	46.5	99	49	46.3	99	50
283	47.7	50	47.4	99	50	47.5	100	49	46.9	98	50
311	48.3	50	47.9	99	50	47.9	99	49	47.8	99	50
339	48.9	50	48.6	100	50	48.9	100	49	48.6	99	50
367	49.0	50	48.4	99	50	49.1	100	48	49.1	100	50
395	50.6	49	50.2	99	50	50.2	99	48	50.7	100	50
423	51.3	48	51.0	99	50	51.2	100	48	51.4	100	50
451	51.1	48	50.6	99	50	50.9	100	48	51.2	100	49
479	51.5	48	50.9	99	50	51.5	100	48	51.7	100	49
507	52.4	47	52.2	100	48	52.1	99	48	52.9	101	49
535	50.9	47	51.4	101	48	51.3	101	47	51.7	102	49
563	51.1	45	51.7	101	47	51.3	100	46	52.2	102	47
591	51.2	45	51.8	101	46	51.9	101	45	52.3	102	45
619	51.2	42	51.1	100	46	51.7	101	44	52.0	102	44
647	51.0	41	51.6	101	42	51.7	101	42	52.0	102	43
675	51.0	39	52.3	103	39	51.4	101	41	51.7	101	40
703	50.0	39	50.8	102	39	51.1	102	38	50.7	102	40
Mean for Weeks											
1-13	30.1		29.7	99		29.5	98		29.5	98	
14-52	44.8		44.1	98		44.4	99		43.9	98	
53-101	50.9		51.1	100		51.2	101		51.5	101	

TABLE 15
Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control		10 mg/m³			30 mg/m³			100 mg/m³		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	19.3	50	18.9	98	50	19.1	99	50	18.9	98	50
10	21.0	50	21.2	101	50	21.1	100	50	21.0	100	50
17	22.2	50	22.5	102	50	22.1	100	50	22.4	101	50
24	23.1	50	23.4	101	50	23.7	102	50	23.4	101	50
31	24.2	50	24.4	101	50	24.5	101	50	24.5	101	50
38	24.6	50	25.2	102	50	25.1	102	50	25.1	102	50
45	25.5	50	26.3	103	50	26.0	102	50	26.4	104	50
52	26.2	50	27.5	105	50	26.2	100	50	27.0	103	50
59	26.9	50	27.8	103	50	27.5	102	50	27.3	101	50
66	27.3	50	28.3	104	50	27.7	101	50	28.1	103	50
73	27.6	50	28.8	104	50	28.4	103	50	28.7	104	50
80	28.4	50	29.3	103	50	29.3	103	50	29.3	103	50
87	28.8	50	30.0	104	50	29.7	103	50	29.8	104	50
115	31.5	50	33.2	106	50	33.1	105	50	32.8	104	50
142	33.3	50	35.3	106	50	35.1	106	50	34.8	105	50
171	35.3	50	37.7	107	50	37.5	106	50	37.6	107	49
199	37.9	50	40.0	106	50	39.6	105	49	39.8	105	49
227	39.5	50	41.7	105	50	41.4	105	49	41.7	106	49
255	41.2	50	43.4	105	50	42.4	103	49	43.3	105	49
283	43.2	50	45.4	105	50	44.7	103	49	45.3	105	49
311	44.5	50	46.1	104	50	45.6	103	49	46.1	104	49
339	45.4	50	47.7	105	50	46.1	102	49	47.5	105	48
367	47.2	49	48.6	103	49	48.1	102	49	48.8	103	48
395	50.7	49	51.9	102	49	51.1	101	49	51.8	102	48
423	52.5	49	53.6	102	49	53.0	101	49	53.2	101	48
451	53.2	49	54.0	102	49	54.1	102	49	54.2	102	48
479	55.4	49	56.3	102	48	55.3	100	49	56.0	101	48
507	56.7	48	57.9	102	48	57.2	101	47	58.3	103	48
535	57.2	47	58.1	101	48	57.5	100	47	57.8	101	47
563	56.6	46	57.4	101	48	57.1	101	47	57.3	101	46
591	57.8	45	57.8	100	45	58.1	101	47	57.1	99	45
619	57.6	43	58.7	102	42	57.9	101	46	57.3	100	44
647	57.9	39	59.3	102	40	57.8	100	45	56.7	98	41
675	56.6	38	57.9	102	40	57.1	101	42	56.0	99	37
703	55.9	36	56.8	102	38	55.0	98	42	52.4	94	33
Mean for Weeks											
1-13	25.0		25.7	103		25.4	102		25.5	102	
14-52	39.1		41.2	105		40.6	104		41.0	105	
53-101	55.0		56.0	102		55.3	101		55.1	100	



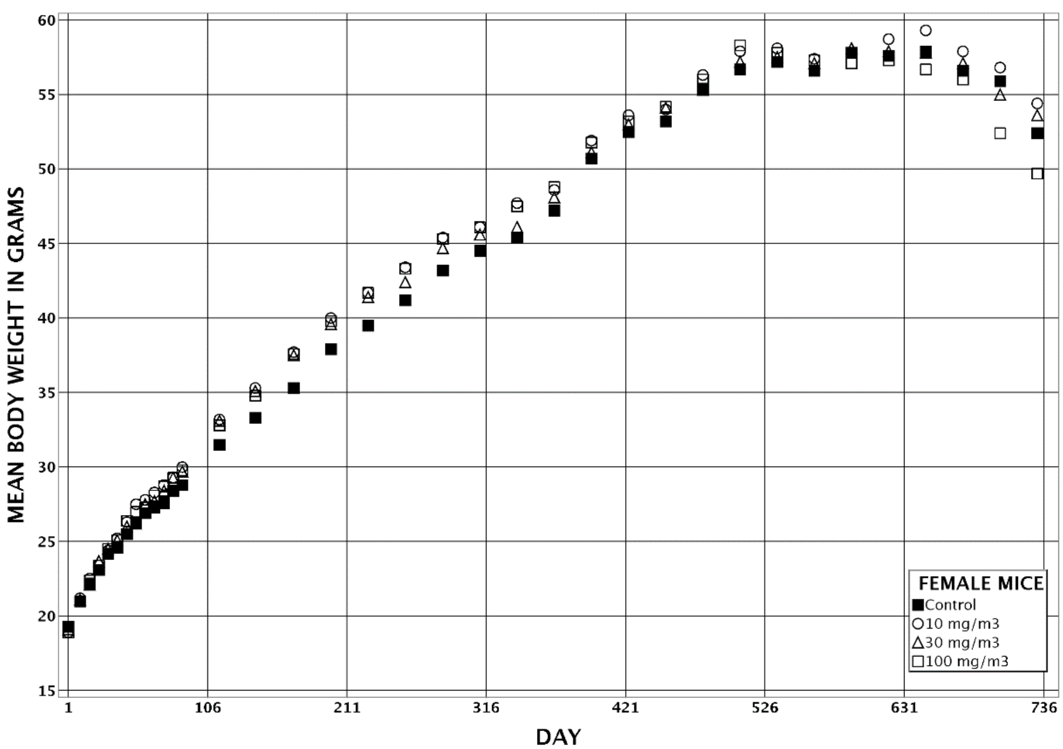


FIGURE 6
Growth Curves for Mice Exposed to TRIM VX by Inhalation for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the lung, nose, larynx, bronchial lymph node, liver, and Harderian gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Lung: In males exposed to 100 mg/m^3, there were significantly increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) and multiple alveolar/bronchiolar carcinoma (Tables 16, C1 and C2). There were also positive trends in the incidences of alveolar/bronchiolar adenoma or carcinoma (combined) and multiple alveolar/bronchiolar carcinoma. The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in the 100 mg/m^3 group exceeded the historical control ranges for inhalation studies and for all routes of administration (Tables 16 and C3). In females exposed to 100 mg/m^3, there were significantly increased incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) (Tables 16 and D2), and positive trends in the incidences of these neoplasms. There was an increased incidence of multiple aveolar/bronchiolar carcinoma in 100 mg/m^3 females, but the incidence was not statistically significant. The incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) in the 100 mg/m^3 females exceeded the historical control ranges for inhalation studies and for all routes of administration (Tables 16 and D3). In addition, a bronchiole epithelium adenoma occurred in one 30 mg/m^3 male; bronchiolar adenomas have not occurred in historical control male B6C3F1/N mice (Tables 16 and C3).

Alveolar/bronchiolar adenomas were densely cellular expansile masses that replaced and sometimes partially compressed adjacent normal alveoli. Most of the masses were located within the alveolar parenchyma (Plate 29), whereas others involved the terminal bronchioles, alveolar ducts, and immediately adjacent alveoli. They were composed of relatively uniform columnar to polygonal cells with moderate amounts of eosinophilic cytoplasm and relatively uniform nuclei with homogeneous chromatin and formed gland-like, solid, or papillary structures (Plate 30). Mitoses were rare.

Alveolar/bronchiolar carcinomas were irregularly shaped, compressive masses that effaced the normal pulmonary parenchyma (Plate 31). Neoplastic cells had a heterogeneous growth pattern consisting of papillary and glandular-like structures and occasionally solid areas. The neoplastic cells were pleomorphic with scant to moderate amounts of eosinophilic cytoplasm and oval to round, hyperchromatic to vesicular nuclei. Nucleoli were occasionally prominent and occasional mitotic figures were seen (Plate 32).

The bronchiole epithelium adenoma was an expansile mass that occurred within a terminal bronchiole (Plate 33). It was a densely cellular mass composed of well-defined, irregular papillary structures lined by one to three layers of relatively uniform, plump, nonciliated cuboidal to low columnar epithelial cells (Plate 34). The toxicological significance of the single incidence of this neoplasm is not known.

There were significantly increased incidences of alveolar/bronchiolar epithelium hyperplasia, infiltration cellular histiocyte, and chronic inflammation in 30 and 100 mg/m^3 males and females (Tables 16, C4, and D4). There were significantly increased incidences of alveolar epithelium hyperplasia in 100 mg/m^3 males and females. There were also significantly increased incidences of fibrosis in 30 and 100 mg/m^3 males and 100 mg/m^3 females. These lesions were qualitatively similar to the nonneoplastic lung lesions observed in rats but were not as severe. They were multifocal to locally extensive and were frequently intermingled and contiguous such that it was often difficult to establish clear demarcations between the various changes. In general, the incidences increased with increasing exposure concentration, and the severities were higher in the 100 mg/m^3 groups than in the other groups.

Alveolar epithelium hyperplasia in mice was morphologically similar to the same lesion in the rat study. They were multifocal, discrete, irregularly shaped, minimal to marked proliferations of epithelial cells within the alveolar parenchyma in which the alveolar architecture was still discernable (Plate 35). Component epithelial cells were well-differentiated, plump, and typically flattened cuboidal cells (Plate 36).

Alveolar/bronchiolar epithelium hyperplasia in mice was also a discrete, multifocal lesion that was similar to the same lesion in the rat lungs and occurred in the periacinar region of the lung. However, in contrast to rats, the proliferating cells formed irregular papillary structures that sometimes projected from the epithelium and filled the lumens of the terminal bronchioles, alveolar ducts, and immediately adjacent septa (Plate 37). A single layer of mostly flat to cuboidal, typically nonciliated epithelial cells lined the papillary structures, but in some areas the

TABLE 16
Incidences of Neoplasms and Nonneoplastic Lesions of the Respiratory System in Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	$10 mg/m^3$	$30 mg/m^3$	$100 mg/m^3$
Male				
Lung[a]	50	50	49	50
Alveolar Epithelium, Hyperplasia[b]	3 (2.0)[c]	3 (1.0)	7 (1.1)	47** (1.7)
Alveolar/bronchiolar Epithelium, Hyperplasia	3 (3.0)	7 (1.4)	15** (1.3)	50** (2.3)
Fibrosis	0	2 (1.0)	5* (1.2)	45** (1.3)
Infiltration Cellular, Histiocyte	5 (2.0)	9 (1.6)	15* (1.5)	49** (2.0)
Inflammation, Chronic	5 (1.4)	12 (1.0)	16** (1.3)	50** (2.4)
Bronchiole, Epithelium, Adenoma[d]	0	0	1	0
Alveolar/bronchiolar Adenoma, Multiple	1	1	0	3
Alveolar/bronchiolar Adenoma (includes multiple)	6	8	5	9
Alveolar/bronchiolar Carcinoma, Multiple	2▲▲	0	2	8*
Alveolar/bronchiolar Carcinoma (includes multiple)	10	8	9	17
Alveolar/bronchiolar Adenoma or Carcinoma[e]				
Overall rate[f]	14/50 (28%)	14/50 (28%)	11/49 (22%)	23/50 (46%)
Adjusted rate[g]	30.5%	30.7%	24.7%	49.4%
Terminal rate[h]	9/38 (24%)	13/39 (33%)	9/37 (24%)	18/37 (49%)
First incidence (days)	549	656	533	571
Poly-3 test[i]	P=0.013	P=0.581	P=0.352N	P=0.047
Nose	49	50	49	50
Exudate	2 (1.0)	11** (1.2)	35** (2.0)	49** (2.5)
Inflammation, Chronic Active	3 (1.0)	33** (1.0)	39** (1.3)	50** (1.5)
Nasopharyngeal Duct, Perforation	0	1	11**	19**
Olfactory Epithelium, Accumulation, Hyaline Droplet	2 (1.0)	46** (1.5)	48** (1.9)	50** (2.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	7 (1.0)	49** (1.9)	49** (1.9)	50** (2.0)
Respiratory Epithelium, Atrophy	0	1 (1.0)	20** (1.0)	40** (1.1)
Respiratory Epithelium, Necrosis	2 (1.0)	1 (1.0)	2 (1.0)	23** (1.1)
Turbinate, Atrophy	0	2 (1.5)	5* (1.0)	14** (1.0)
Turbinate, Perforation	0	0	1	13**
Larynx	48	49	49	49
Epiglottis, Hyperplasia, Squamous	1 (2.0)	2 (1.0)	14** (1.1)	30** (1.2)
Epiglottis, Metaplasia, Squamous	0	49** (2.0)	49** (3.1)	49** (3.9)
Lymph Node, Bronchial	38	37	39	39
Hyperplasia, Lymphoid	3 (1.7)	3 (1.3)	2 (1.0)	14** (1.4)
Infiltration Cellular, Histiocyte	0	1 (1.0)	0	7** (1.6)

TABLE 16
Incidences of Neoplasms and Nonneoplastic Lesions of the Respiratory System in Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Female				
Lung	50	50	50	50
Alveolar Epithelium, Hyperplasia	0	0	2 (1.5)	43** (1.9)
Alveolar/bronchiolar Epithelium, Hyperplasia	0	3 (1.0)	8** (1.1)	45** (2.6)
Fibrosis	0	0	2 (1.0)	42** (1.5)
Infiltration Cellular, Histiocyte	1 (2.0)	4 (2.3)	15** (1.2)	48** (2.3)
Inflammation, Chronic	1 (1.0)	6 (1.2)	26** (1.1)	47** (2.4)
Alveolar/bronchiolar Adenoma, Multiple	0	0	0	2
Alveolar/bronchiolar Adenoma (includes multiple)	4	5	3	8
Alveolar/bronchiolar Carcinoma, Multiple	2	0	1	5
Alveolar/bronchiolar Carcinoma (includes multiple)[j]				
Overall rate	5/50 (10%)	3/50 (6%)	6/50 (12%)	14/50 (28%)
Adjusted rate	11.4%	6.7%	13.1%	31.4%
Terminal rate	5/35 (14%)	2/36 (6%)	6/36 (17%)	8/30 (27%)
First incidence (days)	731 (T)	647	731 (T)	513
Poly-3 test	P<0.001	P=0.342N	P=0.529	P=0.018
Alveolar/bronchiolar Adenoma or Carcinoma[k]				
Overall rate	9/50 (18%)	8/50 (16%)	8/50 (16%)	20/50 (40%)
Adjusted rate	20.2%	17.8%	17.5%	44.5%
Terminal rate	7/35 (20%)	7/36 (19%)	8/36 (22%)	12/30 (40%)
First incidence (days)	575	647	731 (T)	513
Poly-3 test	P<0.001	P=0.491N	P=0.476N	P=0.011
Nose	50	50	50	50
Exudate	8 (1.1)	17* (1.0)	48** (2.0)	49** (2.9)
Inflammation, Chronic Active	4 (1.3)	25** (1.0)	49** (1.2)	49** (1.6)
Nasopharyngeal Duct, Perforation	0	0	14**	17**
Olfactory Epithelium, Accumulation, Hyaline Droplet	14 (1.2)	48** (1.6)	50** (2.0)	50** (2.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	23 (1.2)	50** (1.8)	50** (2.0)	50** (2.6)
Respiratory Epithelium, Atrophy	1 (1.0)	2 (1.0)	28** (1.0)	39** (1.1)
Respiratory Epithelium, Necrosis	0	2 (1.0)	13** (1.0)	23** (1.0)
Turbinate, Atrophy	0	0	10** (1.1)	19** (1.0)
Turbinate, Perforation	0	0	6*	6*
Larynx	50	50	50	50
Epiglottis, Hyperplasia, Squamous	4 (1.0)	3 (1.0)	16** (1.5)	42** (1.6)
Epiglottis, Metaplasia, Squamous	0	50** (2.4)	50** (3.5)	50** (4.0)
Lymph Node, Bronchial	44	44	44	43
Hyperplasia, Lymphoid	6 (1.3)	4 (1.0)	9 (1.4)	9 (2.0)
Infiltration Cellular, Histiocyte	1 (1.0)	0	2 (1.0)	4 (2.0)

TABLE 16
Incidences of Neoplasms and Nonneoplastic Lesions of the Respiratory System in Mice in the 2-Year Inhalation Study of TRIM VX

* Significantly different ($P\leq0.05$) from the chamber control group by the Poly-3 test
** $P\leq0.01$
▲▲ Significant ($P\leq0.01$) Poly-3 trend test
(T) Terminal kill
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 0/250; all routes: 0/550
[e] Historical incidence for inhalation studies: 69/250 (27.6% ± 2.6%), range 26%-32%; all routes: 147/550 (26.7% ± 6.5%), range 16%-38%
[f] Number of animals with neoplasm per number of animals with lung examined microscopically
[g] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[h] Observed incidence at terminal kill
[i] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in an exposure group is indicated by **N**.
[j] Historical incidence for inhalation studies: 17/249 (6.8% ± 3.7%), range 2%-10%; all routes: 24/549 (4.4% ± 3.5%), range 0%-10%
[k] Historical incidence for inhalation studies: 28/249 (11.3% ± 5.5%), range 6%-18%; all routes: 50/549 (9.1% ± 5.2%), range 2%-18%

cells were polygonal or low cuboidal (Plate 38). Also in contrast to rats, the proliferations in the terminal bronchioles of mice occurred more proximally in mice.

Inflammation chronic was a multifocal to coalescing lesion of minimal to mild severity characterized by irregular areas in which the alveolar architecture was obscured by dense aggregates of mostly macrophages mixed with neutrophils, lymphocytes, and cellular debris. The lesions were often more prominent in perivascular areas and, in these areas, the lymphocytes component was the most prominent component. Variable epithelial cell hyperplasia and minimal to mild fibrosis of the alveolar septal interstitium was a consistent component of the areas of inflammation. It was often difficult to distinguish denser inflammatory cell accumulation from hyperplastic epithelium. Infiltration cellular histiocyte was composed of minimal to mild accumulations of large macrophages with foamy to amphophilic cytoplasm present in the surrounding alveoli.

Nose: In all exposed groups of males and females, there were significantly increased incidences of exudate, chronic active inflammation, and olfactory epithelium hyaline droplet accumulation (Tables 16, C4, and D4). In the respiratory epithelium, there were significantly increased incidences of hyaline droplet accumulation in all exposed groups of males and females, atrophy in 30 and 100 mg/m^3 males and females, and necrosis in 100 mg/m^3 males and 30 and 100 mg/m^3 females. The incidences of turbinate atrophy were significantly increased in 30 and 100 mg/m^3 males and females, and the incidences of turbinate perforation were significantly increased in 100 mg/m^3 males and 30 and 100 mg/m^3 females. There were also significantly increased incidences of nasopharyngeal duct perforation in 30 and 100 mg/m^3 males and females.

Exudate consisted of minimal to marked accumulations of dense proteinaceous fluid and/or concretions of amorphous, eosinophilic, hyaline material in the ventral nasal passages of the ethmoid region of the nose (Plate 39). The exudate contained variable numbers of neutrophils and cellular debris. Chronic active inflammation was characterized by the presence of mild to moderate infiltrates of macrophages, lymphocytes, and neutrophils within the submucosa adjacent to the areas of exudate. Nasopharyngeal duct perforation was characterized by rupture of the wall of the nasopharyngeal duct associated with the exudate in the nasal passages (Plate 39). Turbinate perforation, characterized by focal necrosis and discontinuity of the ventral ethmoid turbinates, was primarily associated with the exudate in the nasal passages. Both lesions most likely resulted from pressure necrosis caused by exudate. Turbinate atrophy was observed in the ethmoid turbinates and was also associated with the nasal exudate; affected turbinates were thin, short, and blunt (Plate 39).

Hyaline droplet accumulation in the respiratory and olfactory epithelia was morphologically similar to this lesion in rats. Respiratory epithelial atrophy was a focal minimal change associated with areas of exudate. In affected sites, the height of the epithelium decreased due to replacement of the normally present cuboidal to columnar sometimes ciliated epithelial cells by a single layer of flattened to low cuboidal epithelial cells. Necrosis of the respiratory epithelium in the ventral aspects of the ethmoid region was also associated with exudate and consisted of focal areas of denudation and loss of small focal areas of the epithelium (Plate 39).

Larynx: In the epiglottis, there were significantly increased incidences of squamous hyperplasia in 30 and 100 mg/m^3 males and females and squamous metaplasia in all exposed groups of males and females (Tables 16, C4, and D4). These lesions were morphologically similar to those observed in the larynx of male and female rats.

Bronchial Lymph Node: In 100 mg/m^3 males, there were significantly increased incidences of lymphoid hyperplasia and infiltration cellular histiocyte (Tables 16 and C4). Lymphoid hyperplasia was characterized by enlargement of the affected lymph nodes due to increased numbers of well-differentiated small lymphocytes mixed with focal, multifocal to coalescing aggregates of plump macrophages with abundant, foamy, pale amphophilic cytoplasm. These lesions were morphologically similar to the changes that were observed in the bronchial and mediastinal lymph nodes of male and female rats and diagnosed as lymphohistiocytic hyperplasia. However, the changes were diagnosed separately because lymphoid hyperplasia sometimes occurred in the absence of histiocytic aggregates.

Liver: There was a positive trend in the incidences of hepatocellular adenoma in males and the incidence in the 100 mg/m^3 group was significantly increased (chamber control, 23/50; 10 mg/m^3, 29/50; 30 mg/m^3, 26/50; 100 mg/m^3, 36/50; Table C2). However, the incidence in the 100 mg/m^3 group was within the historical control ranges for inhalation studies [142/250 (56.8% ± 11.2%); range 46%-74%] and all routes [328/550 (59.6% ± 11.2%); range 46%-78%], and the significantly increased incidence was attributed to the uncommonly low incidence in the chamber control group. A few hepatoblastomas occurred in 30 and 100 mg/m^3 males but the incidences were not statistically significant. In females, there was a positive trend in the incidences of hepatocellular carcinoma (7/50, 5/50, 7/50, 12/50; Table D2) and the incidence in the 100 mg/m^3 group exceeded the historical control ranges for inhalation studies [41/250 (16.4% ± 3.6%); range 12%-20%] and all routes [76/549 (13.9% ± 5.2%); range 4%-20%]. However, the incidence of hepatocellular carcinoma in the 100 mg/m^3 group was not statistically significant relative to that of the concurrent chamber controls and was not considered to be related to treatment. The combined incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma in both sexes were within the ranges observed in historical control animals. Tumors were morphologically similar to those that occur spontaneously.

Harderian Gland: In females, there was a positive trend in the incidence of adenoma or carcinoma (combined) (4/50, 3/50, 7/50, 9/50; Table D2). The incidence in the 100 mg/m^3 group exceeded the historical control range for inhalation studies [20/250 (8.0% ± 3.7%); range 4%-14%]. However, the incidence of adenoma or carcinoma (combined) was not statistically significant relative to that of the concurrent chamber controls and was not considered to be related to treatment.

GENETIC TOXICOLOGY

TRIM VX (dose range tested, 500 to 10,000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA98 or TA100 or in *Escherichia coli* strain WP2 *uvrA*/pKM101 in the presence or absence of exogenous metabolic activation (S9) (Table E1).

In vivo, no increases in the frequencies of micronucleated reticulocytes or erythrocytes were observed in peripheral blood samples from male or female Wistar Han rats or B6C3F1/N mice exposed to TRIM VX via inhalation (25 to 400 mg/m^3) for 3 months (Tables E2 and E3).

In addition to the micronucleus endpoint, the percentage of reticulocytes among circulating red blood cells was calculated as a measure of bone marrow toxicity or perturbations in erythropoiesis (Tables E2 and E3). The very small increases in percent reticulocytes noted in male rats and female mice were within historical ranges for this endpoint and, in the absence of any observed hematological effects, were not considered biologically significant.

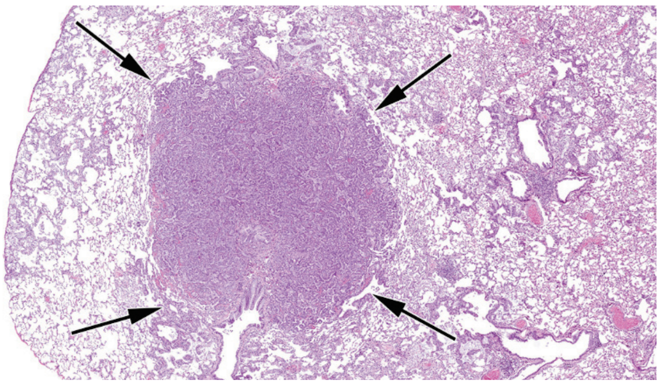


PLATE 1
Alveolar/bronchiolar adenoma in the lung of a female Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. The adenoma (arrows) appears as a densely cellular, nodular mass that is distinctly demarcated from the surrounding alveolar parenchyma. H&E

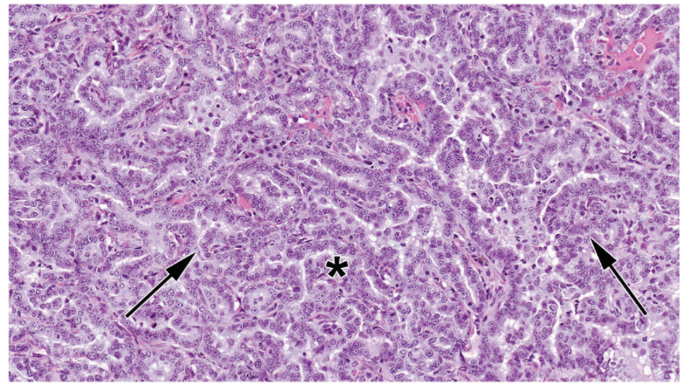


PLATE 2
Higher magnification of Plate 1. The adenoma is composed of a generally uniform population of cuboidal epithelial cells forming relatively well-defined papillary (arrows) and glandular (asterisk) structures that are in some areas separated by thin bands of connective tissue. H&E

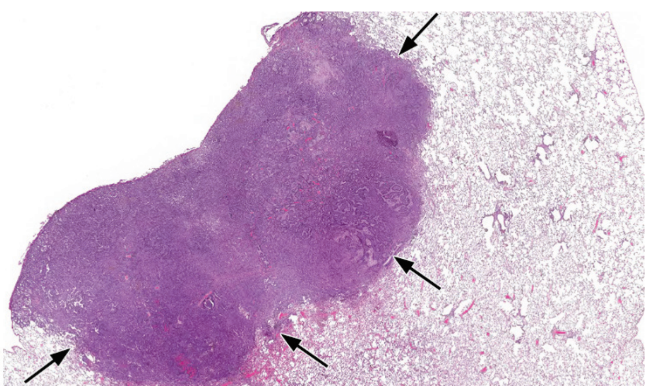


PLATE 3
Alveolar/bronchiolar carcinoma in the lung of a male Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. The carcinoma is highly invasive (arrows) and has effaced the lung parenchyma. H&E

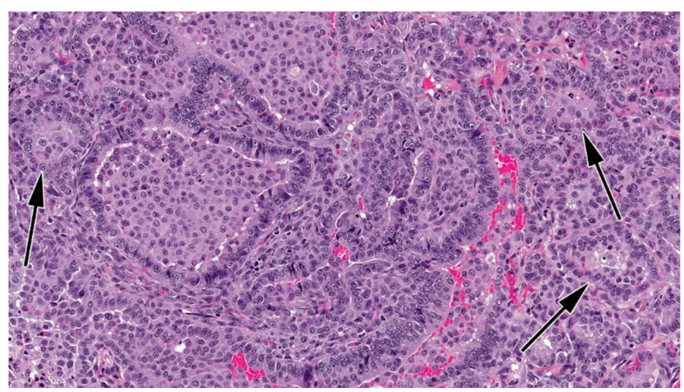


PLATE 4
Higher magnification of Plate 3. There is moderate cellular pleomorphism. The neoplastic cells are mostly cuboidal to columnar and form poorly defined glandular structures (arrows) irregularly separated by thin connective tissue septae; in some areas, the cells are polygonal and densely aggregated. The cells have moderately abundant eosinophilic cytoplasm and variably sized round to elongate nuclei. H&E

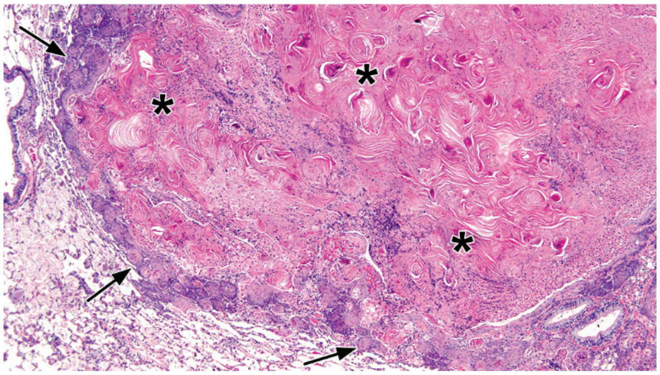


Plate 5
Cystic keratinizing epithelioma in the lung of a female Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. This neoplasm is characterized by a central mass of keratin (asterisks) surrounded by a wall of squamous epithelium (arrows). H&E

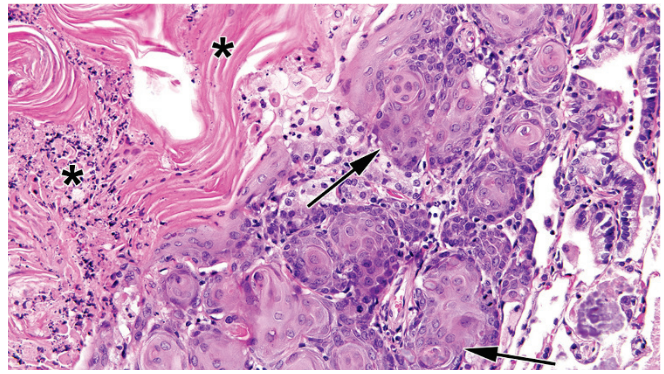


Plate 6
Higher magnification of Plate 5. Note central keratin (asterisks) surrounded by well-differentiated squamous epithelium (arrows). H&E

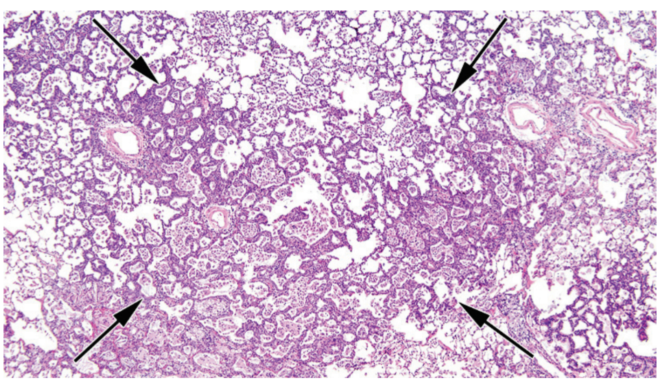


Plate 7
Discrete area of alveolar epithelium hyperplasia (arrows) in the lung of a female Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. The alveolar septae are hypercellular and the architecture of the alveolar parenchyma is generally maintained. H&E

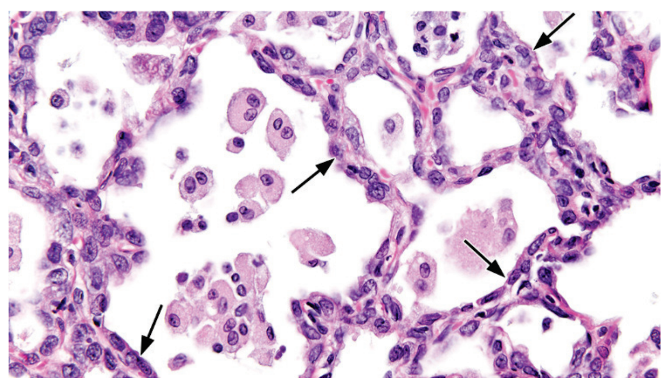


Plate 8
Higher magnification of Plate 7. The alveolar septae are lined by flattened to low cuboidal epithelial (Type II) cells (arrows). Note macrophages within the alveolar spaces. H&E

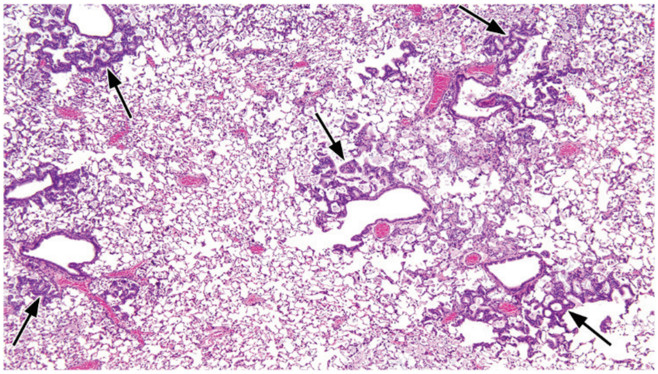


Plate 9
Multifocal areas of alveolar/bronchiolar epithelium hyperplasia (arrows) in the lung of a female Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. The lesions are centered on the terminal bronchioles, alveolar ducts, and immediately adjacent alveoli. H&E

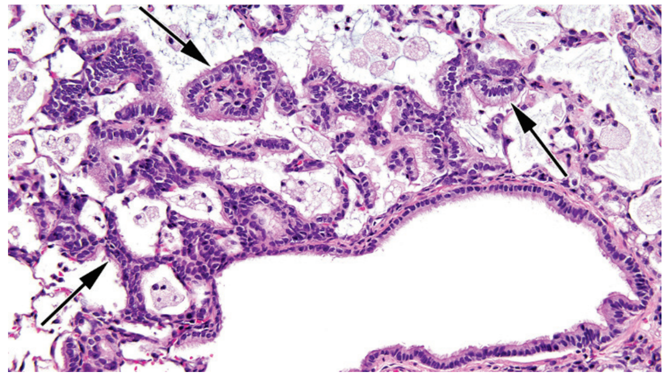


Plate 10
Higher magnification of Plate 9. The hyperplastic cells lining the alveoli adjacent to the alveolar duct are mostly low cuboidal to columnar and ciliated and nonciliated epithelial cells (arrows). H&E

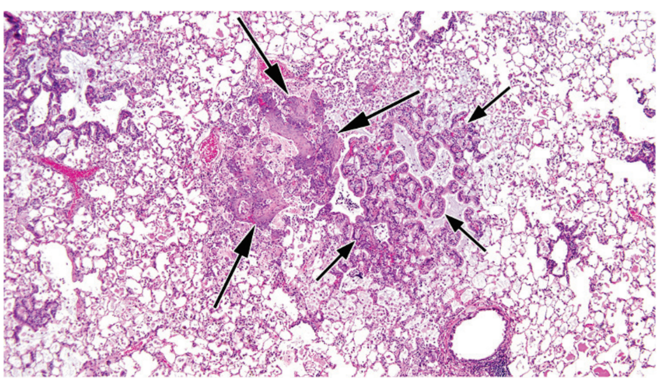


Plate 11
Focal area of alveolar epithelium squamous metaplasia (long arrows) associated with alveolar epithelium hyperplasia (short arrows) in the lung of a female Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. H&E

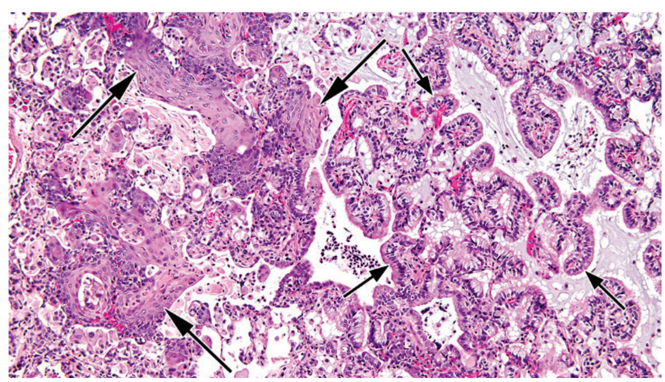


Plate 12
Higher magnification of Plate 11. Squamous metaplasia is characterized by irregular thickening of the alveolar septae by well-differentiated squamous epithelium (long arrows) and is adjacent to alveolar epithelium hyperplasia (short arrows) in which the alveolar septae are lined by ciliated columnar epithelium. Note numerous macrophages, cellular debris, and mucus within the alveolar spaces. H&E

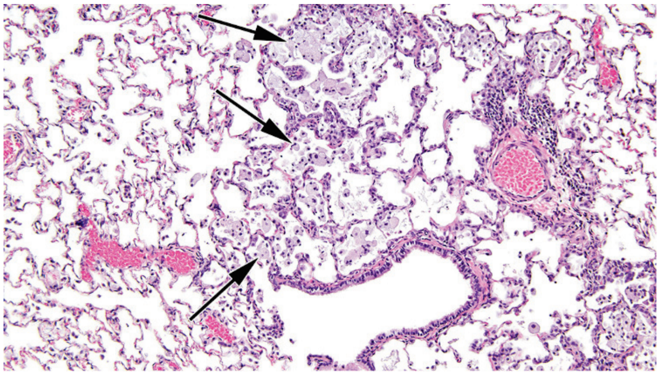


PLATE 13
Infiltration cellular, histiocyte (arrows) within an area of alveolar epithelium hyperplasia in the lung of a male Wistar Han rat exposed to 30 mg/m^3 TRIM VX by inhalation for 2 years. H&E

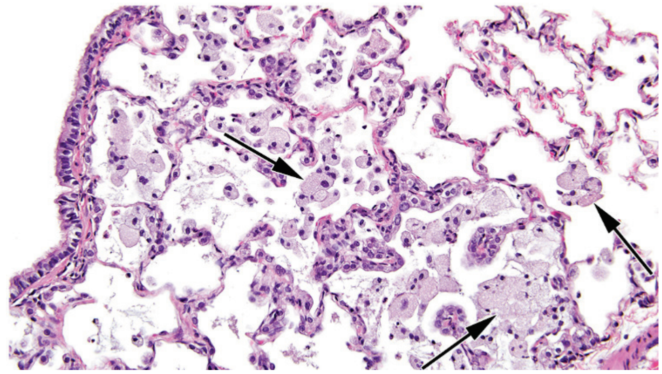


PLATE 14
Higher magnification of Plate 13. The alveoli contain numerous swollen macrophages (histiocytes) (arrows) with foamy eosinophilic cytoplasm. H&E

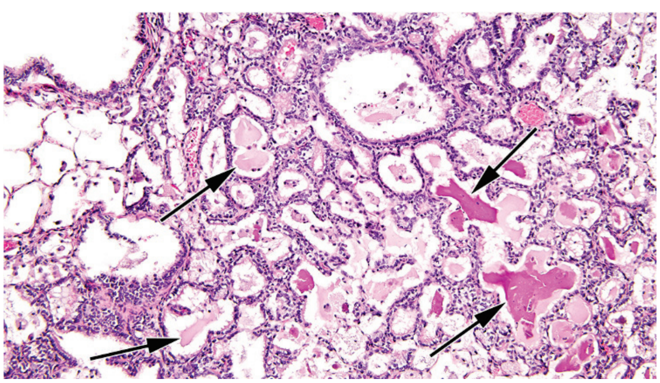


PLATE 15
Alveolus proteinosis in the lung of a female Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. Alveoli within an area of alveolar epithelial hyperplasia contain pale or brightly eosinophilic protein material (arrows). H&E

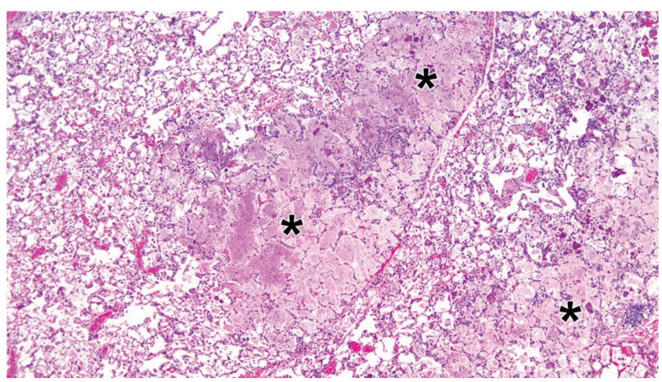


PLATE 16
Alveolus proteinosis in the lung of a female Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. Alveoli within an area of inflammation are filled by granular-appearing eosinophilic protein material (asterisks). H&E

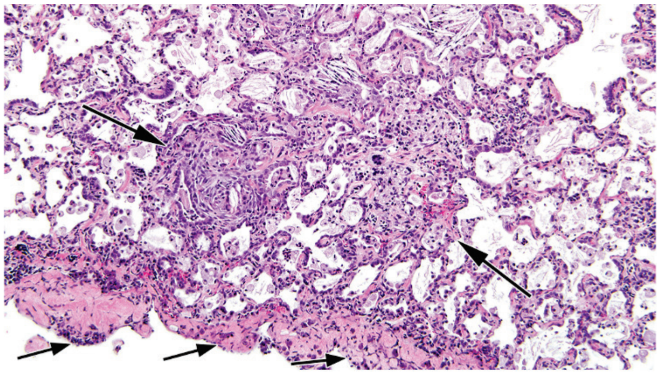


Plate 17
An area of marked chronic active inflammation (long arrows) in the lung of a male Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. The alveoli are filled mostly with macrophages mixed with lesser numbers of neutrophils, degenerate cellular debris, and a few multinucleated giant cells. Note clear, angular cleft-like spaces (cholesterol clefts) among the inflammatory cells and debris. The pleura is irregularly thickened by marked fibrosis (short arrows). H&E

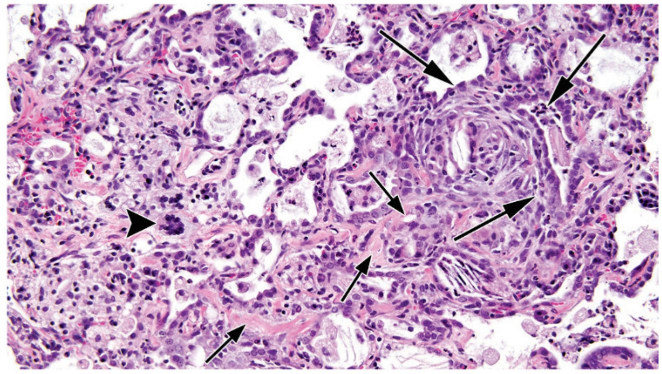


Plate 18
Higher magnification of Plate 17. In addition to the inflammatory cell infiltrates, there is alveolar epithelium hyperplasia (long arrows) and irregular fibrosis of the alveolar septae (short arrows). Note multinucleated giant cells (arrowhead). H&E

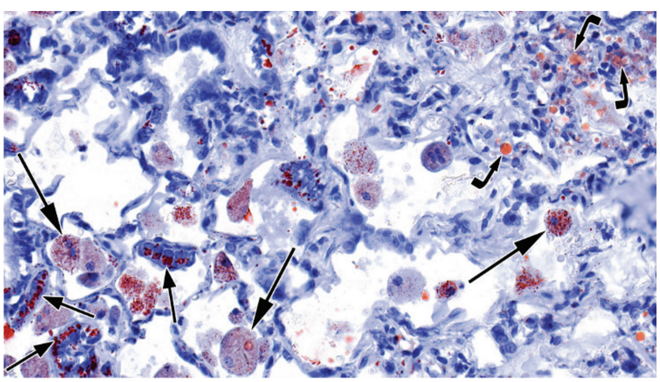


Plate 19
Section of the lung of a female Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. Note oil droplets within macrophages (long arrows), within the hyperplastic alveolar epithelium (short arrows), and free within the alveolar spaces (curved arrows). Oil-red-O

Plate 20
Lymphohistiocytic hyperplasia in the bronchus-associated lymphoid tissue (BALT) in the lung of a female Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. The BALT is markedly expanded by increased numbers of lymphocytes (long arrows) and macrophages (short arrows). H&E

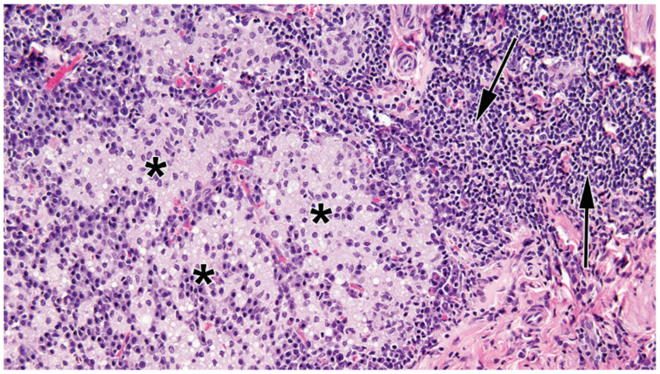


PLATE 21
Higher magnification of Plate 20. Note areas of well-differentiated lymphocytes (arrows) and macrophages swollen with abundant lightly eosinophilic foamy cytoplasm (asterisks) with scattered low numbers of plasma cells. H&E

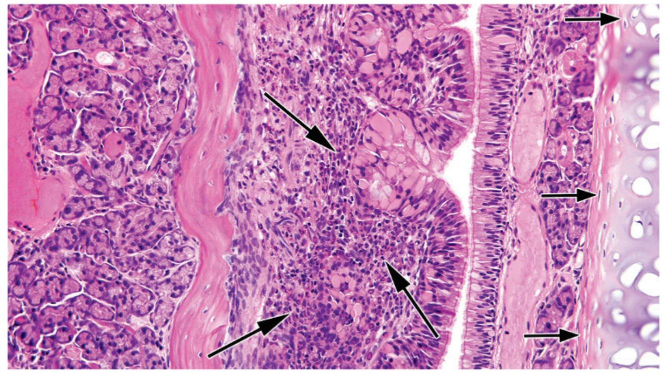


PLATE 22
Suppurative inflammation in a nasal maxilloturbinate in the Level II section of the nose of a female Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. There are numerous neutrophils within the submucosal tissue of the turbinate (long arrows) adjacent to the nasal septum (short arrows). H&E

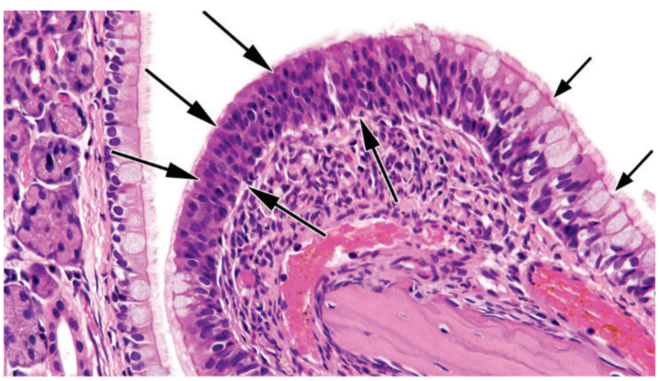


PLATE 23
Focal area of respiratory epithelium hyperplasia at the tip of a maxilloturbinate in the nose of a female Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. In contrast to the normal pseudostratified, ciliated, tall columnar epithelium (short arrows) lining the remainder of the turbinate and the lateral wall, the affected segment of the epithelium appears hypercellular with disorganized piling up of the epithelial cells (long arrows). H&E

PLATE 24
Transitional epithelium hyperplasia in the nose of a female Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. Note the disorganized piling up of the epithelial cells (arrows). Normal transitional epithelium lining the lateral wall is composed of a single row of ciliated, cuboidal to columnar epithelial cells. H&E

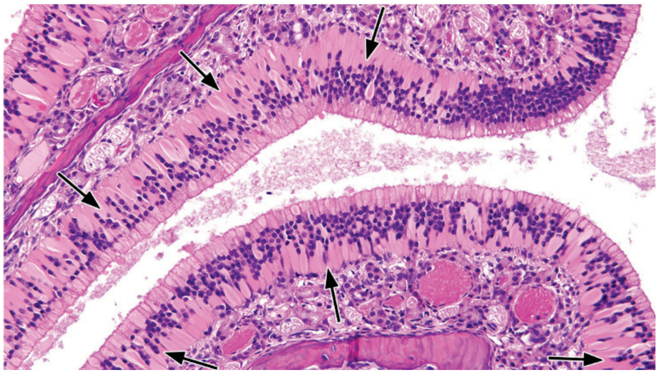


PLATE 25
Hyaline droplet accumulation in the olfactory epithelium in the nose of a male Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. The olfactory epithelium is distorted due to distention of the epithelial cells by intracytoplasmic accumulation of brightly eosinophilic, homogenous, globular material (arrows) that partially or completely filled the cytoplasm. H&E

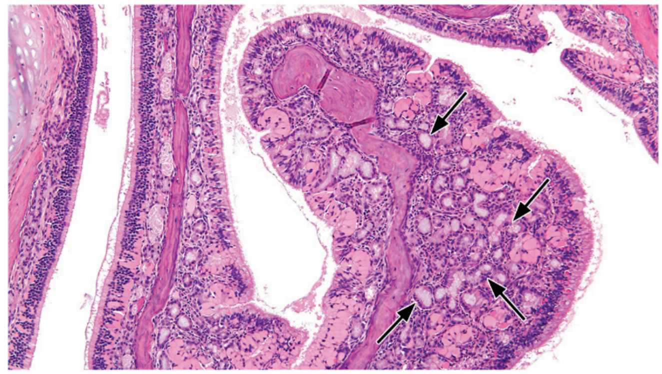


PLATE 26
Hyperplasia of the submucosal glands in the olfactory epithelium of the ventral ethmoid turbinates in the nose of a male Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. There are increases in the size of the glands and number of gland profiles (arrows). Note hyaline droplet accumulation within the olfactory epithelial cells. H&E

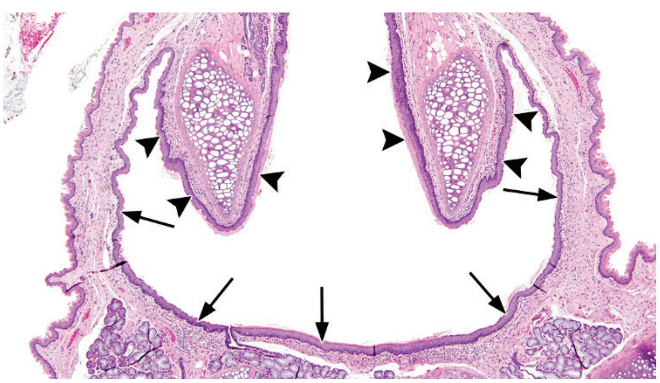


PLATE 27
Squamous metaplasia in the larynx of a male Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. Squamous metaplasia is characterized by replacement of the cuboidal to low columnar, ciliated epithelium that normally lines the base and lateral aspects of the epiglottis by multiple layers of well-differentiated, variably keratinized squamous epithelium (arrows). Squamous hyperplasia was characterized by thickening of the squamous epithelium that normally lines the arytenoid cartilages (arrowheads). H&E

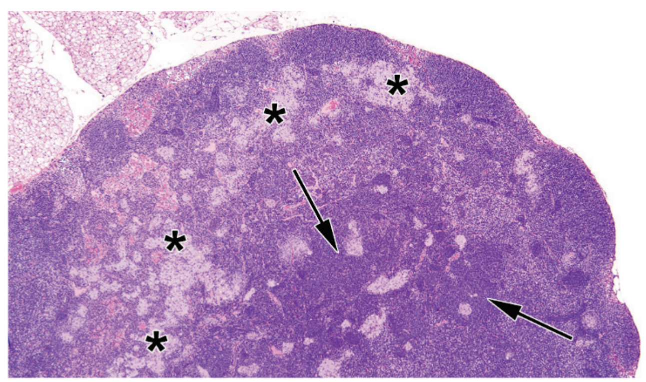


PLATE 28
Lymphohistiocytic hyperplasia in the bronchial lymph node of a male Wistar Han rat exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. The lymph node is markedly expanded by increased numbers of lymphocytes (arrows) and macrophages (asterisks). H&E.

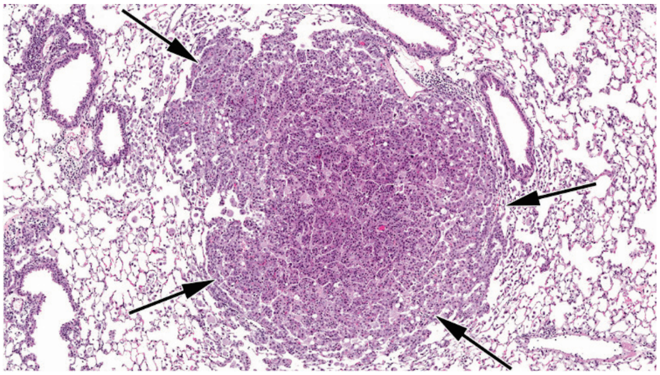


Plate 29
Alveolar/bronchiolar adenoma in the lung of a female B6C3F1/N mouse exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. The adenoma (arrows) is distinctly demarcated from the surrounding alveolar parenchyma. H&E

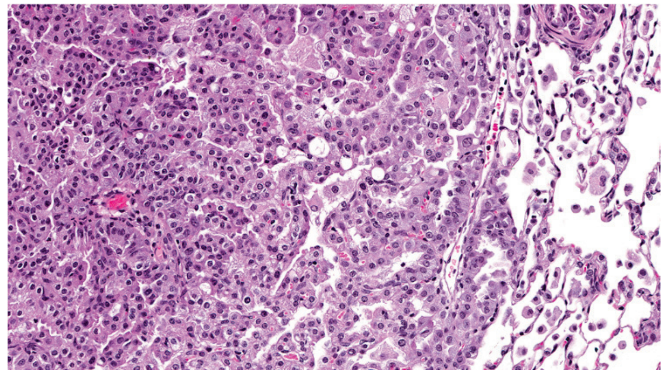


Plate 30
Higher magnification of Plate 29. Note that the neoplastic cells form poorly and well-defined papillary and glandular structures. H&E

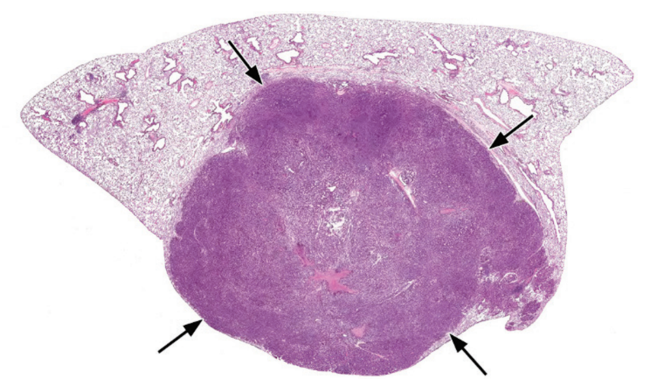


Plate 31
Alveolar/bronchiolar carcinoma in the lung of a male B6C3F1/N mouse exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. The carcinoma (arrows) is a densely cellular, well-demarcated mass that has effaced a large portion of the lung and compresses the immediately adjacent lung parenchyma. H&E

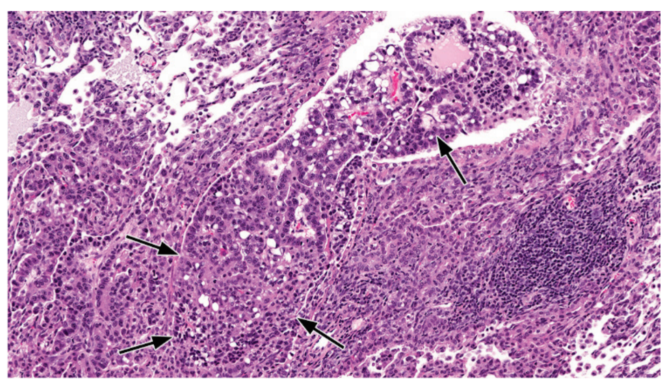


Plate 32
Higher magnification of Plate 31. The carcinoma is composed of neoplastic cells that form poorly defined papillary structures. Note that the neoplasm has invaded the epithelium lining the terminal bronchiole and has grown into the lumen (arrow). H&E

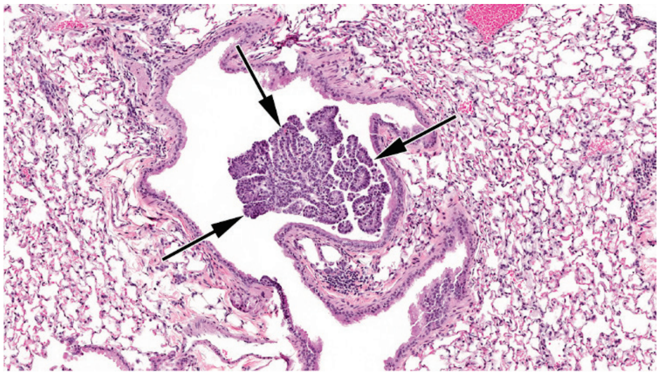


PLATE 33
Bronchiole epithelium adenoma in the lung of a male B6C3F1/N mouse exposed to 30 mg/m^3 TRIM VX by inhalation for 2 years. The neoplasm is an exophytic mass (arrows) within the bronchial lumen and is composed of irregular papillary structures. H&E

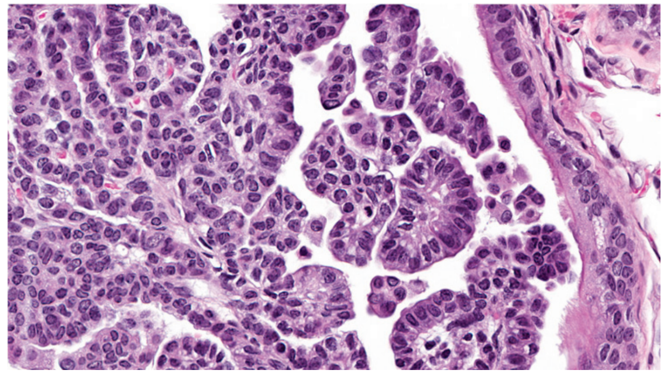


PLATE 34
Higher magnification of Plate 33. The neoplastic cells vary from cuboidal to columnar and form irregular papillary structures supported by a core of scant connective stroma. H&E

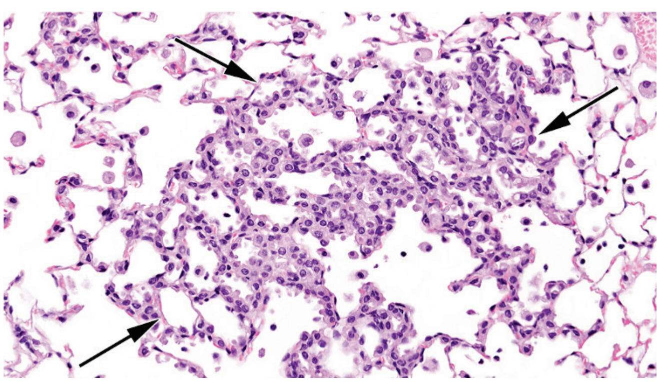


PLATE 35
Focal alveolar epithelium hyperplasia in the lung of a male B6C3F1/N mouse exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. In the area of hyperplasia (arrows), the alveolar septae appear hypercellular in contrast to the surrounding normal alveolar septae. The alveolar architecture is generally maintained. H&E

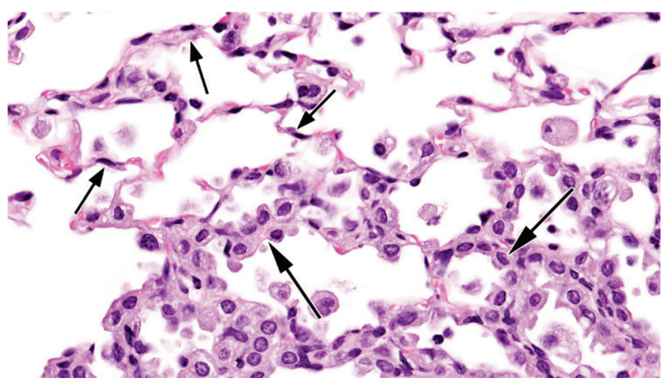


PLATE 36
Higher magnification of Plate 35. Alveolar septae are hypercellular and lined by cuboidal to polygonal cells (long arrows). Note adjacent normal-appearing alveolar septae (short arrows). H&E

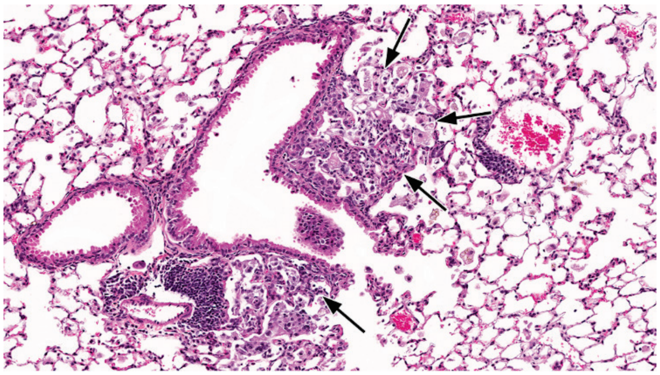


Plate 37
Alveolar/bronchiolar epithelium hyperplasia in the lung of a male B6C3F1/N mouse exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. Focal areas of proliferating epithelial cells (arrows) are adjacent to a terminal bronchiole. Note numerous macrophages and proteinaceous material within the alveoli. H&E

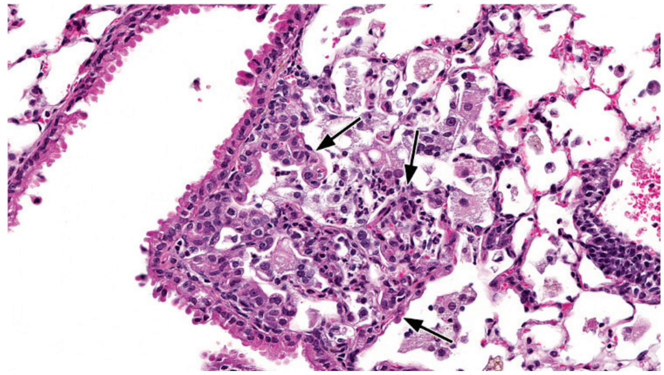


Plate 38
Higher magnification of Plate 37. In the areas of hyperplasia, the alveolar septae are lined by cuboidal to polygonal cells (arrows). H&E

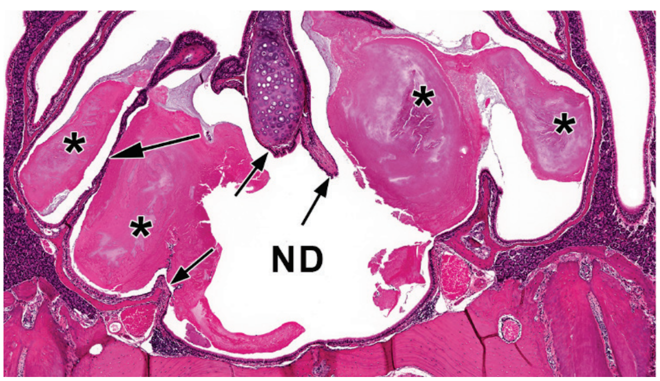


Plate 39
Exudate in the ventral nasal passages in the ethmoid region of the nose of a female B6C3F1/N mouse exposed to 100 mg/m^3 TRIM VX by inhalation for 2 years. The exudate in the ventral nasal passages is deeply eosinophilic and amorphous (asterisks) and is associated with the ruptured walls (short arrows) of the nasopharyngeal duct (ND) and an atrophic ethmoid turbinate (long arrow). H&E

DISCUSSION AND CONCLUSIONS

TRIM VX is a metalworking fluid used as a lubricant and coolant liquid and for cleaning tools and parts during machining operations. Occupational exposures to metalworking fluids occur primarily by dermal exposure and inhalation. Inhalation exposure to metalworking fluids has been reported to cause respiratory problems and to be associated with cancer. Metalworking fluids were nominated by the National Institute for Occupational Safety and Health (NIOSH) for study by the National Toxicology Program (NTP). In addition, NIOSH aided in the selection of metalworking fluids for inhalation toxicity testing by the NTP, which was based on a combination of considerations including high production volumes and commercial availability, chemical composition, and class. In all, nine metalworking fluids underwent chemical analysis and genetic toxicity assessment prior to selection of four formulations for 3-month inhalation studies: CIMSTAR 3800 (semisynthetic), TRIM VX (soluble oil), Syntilo 1023 (synthetic), and TRIM SC210 (semisynthetic). In all four studies, the respiratory tract was the primary site of toxicity in rats and mice (Ryan *et al*., 2016). Based on the respiratory tract toxicity observed in these 3-month studies, CIMSTAR 3800 and TRIM VX were selected for 2-year studies. The findings from the 3-month and 2-year studies with CIMSTAR 3800 have been previously reported (NTP, 2015). The current Technical Report presents the findings of the 3-month and 2-year inhalation studies of TRIM VX in Wistar Han rats and B6C3F1/N mice.

The exposure concentrations (25 to 400 mg/m^3) used in the 3-month studies of the four metalworking fluids, including TRIM VX, were selected based on their low toxicity in previously conducted 3-month studies and because generation of higher concentrations was not feasible due to the high water content of the aerosols. TRIM VX, like many other metalworking fluids, is diluted with water before use and workers are typically exposed to aerosols composed primarily of water. Because it is technically difficult to generate and expose animals to liquid aerosols containing high water content, the metalworking fluid aerosols in the NTP studies were generated from undiluted concentrates and diluted with clean air to produce the desired concentrations. Thus, the exposure concentrations used in these studies were considerably higher than those encountered in an occupational setting. The NIOSH recommended exposure limit for total particulate mass is 0.5 mg/m^3 of metalworking fluid mist, and mists are regulated as a nuisance dust by the Occupational Safety and Health Administration with a permissible exposure limit of 15 mg/m^3 (OSHA, 2001).

In the 3-month studies of TRIM VX, there was no exposure-related mortality in rats or mice. Minor body weight decreases occurred in exposed groups of male rats and mice and the decreases were significant at the highest exposure concentration (400 mg/m^3); body weights of females from both species were similar to those of the chamber controls. Biologically relevant increases in organ weights were recorded in groups exposed to 50 mg/m^3 or greater and included the liver of male and female rats and mice, the lung of female rats and male and female mice, and the spleen of male and female mice. However, corresponding histologic changes were observed only in the lungs.

The respiratory tract was the primary site of toxicity in rats and mice following TRIM VX inhalation exposure for 3 months. In the lung, nonneoplastic lesions including chronic active inflammation, minimal to mild fibrosis, and infiltration of histiocytes generally occurred in both male and female rats and mice. Minimal to mild bronchiole hyperplasia occurred in the lung of male and female mice. Such lesions are commonly observed in the lungs of rats and mice following inhalation exposure to irritant gases, vapors, and relatively insoluble particulates. Previous studies of metalworking fluid have also found that the lung is a primary target for acute and subchronic toxicity in experimental animals (Selgrade *et al*., 1990; Dalbey, 2001; DeLorme *et al*., 2003; Lim *et al*., 2005a,b,c). A number of commonly reported histologic changes in the lungs of rodents exposed to metalworking fluids include the accumulation of macrophages, infiltration of inflammatory cells, and thickening of alveolar walls (Selgrade *et al*., 1990; Dalbey, 2001; Lim *et al*., 2005c). Fibrosis can be progressive and may result in lung disease, such as interstitial lung disease, characterized by scarring (fibrosis) of the alveolar walls. Interstitial lung disease observed in metalworking fluid workers is often attributed to bacteria, endotoxins, and fungi present in the used fluids (DeLorme *et al*., 2003; Lim *et al*., 2005b,c; Tillie-Leblond *et al*., 2011). However, the TRIM VX used in the current study did not contain these contaminants, indicating that fibrosis may be caused by the chemical constituents or physical properties of TRIM VX.

In the nasal cavity, rats had a greater spectrum of lesions than mice. Hyaline droplet accumulation of the olfactory and respiratory epithelium and suppurative inflammation occurred in all exposed groups of male and female rats and mice. Exposed male and female rats also had hyperplasia and squamous metaplasia of the respiratory epithelium and goblet cell hyperplasia in the nose. Similar lesions have been observed in the nasal cavity of rodents following inhalation exposure to gases, vapors, and particulates. Inflammation and epithelial hyperplasia that is most likely regenerative may result from irritation and damage to the nasal epithelium (Haschek *et al*., 2010), and goblet cell hyperplasia and hyaline droplet accumulation are likely adaptive protective responses. Nasal symptoms, such as rhinitis (inflammation of the nasal passages), are common among workers exposed to various types of metalworking fluids including soluble oils (Rosenman *et al*., 1997; Oudyk *et al*., 2003; Park *et al*., 2008; Jaakkola *et al*., 2009). Rhinitis may appear in conjunction with other airway diseases such as asthma, which is also reported in metalworking fluid workers (Kriebel *et al*., 1997; Guerra *et al*., 2002).

There were also exposure-related lesions noted in the larynx such as significantly increased incidences of mild to marked squamous metaplasia, minimal to mild squamous hyperplasia, and chronic inflammation in male and female rats and mice. Squamous metaplasia occurred primarily at the base of the epiglottis, an area that normally consists of ciliated columnar epithelium, and when injured, these cells are often replaced by the more resistant squamous epithelium. Squamous metaplasia of the larynx has been reported in 30% to 40% of inhalation studies conducted in rodents (Kaufmann *et al*., 2009). Mild squamous metaplasia of the larynx is considered an adaptive or protective response to repeated irritation of the rodent epithelium, whereas diffuse moderate to severe metaplasia is considered an adverse effect (Kaufmann *et al*., 2009). Only a few NTP inhalation studies, including diethylamine (NTP, 2011), triethylamine (NTP, 2016e), and CIMSTAR 3800 (NTP, 2015), have shown similar marked squamous metaplasia of the larynx.

Overall, the incidences and severities of nonneoplastic lesions were similar between male and female rats and mice in the 3-month studies. These findings are comparable with the previously conducted 3-month inhalation studies of CIMSTAR 3800, TRIM SC210, and Syntilo 1023 (Ryan *et al*., 2016). However, the lung fibrosis identified at exposure concentrations as low as 50 mg/m^3 in rats and 100 mg/m^3 in mice was specific to TRIM VX exposure. Based on the severity of lung fibrosis in rats and mice, which is considered a secondary response to the observed inflammatory changes, 100 mg/m^3 was chosen as the highest exposure concentration for the 2-year studies with TRIM VX. This exposure concentration was also selected to allow comparison to the 2-year studies of CIMSTAR 3800 (NTP, 2015).

In the 2-year studies, the survival of male and female rats and mice exposed to 10, 30, or 100 mg/m^3 TRIM VX was similar to that of the respective chamber controls. Mean body weights of male and female rats and mice were also similar to those of the chamber controls. As with the 3-month studies of TRIM VX, the respiratory tract was the primary target for toxicity in the 2-year studies.

In male rats exposed for 2 years, there was equivocal evidence of TRIM VX carcinogenicity in the lungs. This conclusion was based on the occurrences of one alveolar/bronchiolar adenoma and two alveolar/bronchiolar carcinomas in the 100 mg/m^3 group. There was a positive trend in the incidences of alveolar/bronchiolar carcinoma, as well as a positive trend in the combined incidences of alveolar/bronchiolar adenoma or carcinoma. Alveolar/bronchiolar carcinomas are uncommon for the Wistar Han rat strain: the incidence of carcinoma (2/50) in the 100 mg/m^3 group exceeded the historical control incidences for inhalation studies (0/150) and all routes (0/299). The combined incidence of alveolar/bronchiolar adenoma or carcinoma (3/50) in the 100 mg/m^3 group was at the upper end of the historical control ranges for inhalation studies (0% to 6%) and all routes (0% to 6%). In addition, the incidences of alveolar epithelium hyperplasia and alveolar/bronchiolar epithelium hyperplasia were significantly increased ($P<0.001$) in all exposed groups of male rats. The severities of these nonneoplastic lesions increased slightly with increasing exposure concentration and may have contributed to the development of adenoma and carcinoma in the lung. Taken together, the low occurrence of these tumors was considered to be equivocal evidence of carcinogenic activity.

In female rats exposed to TRIM VX for 2 years, there was equivocal evidence of carcinogenic activity in the lung. This conclusion was based on the low incidence of alveolar/bronchiolar adenoma in the 100 mg/m^3 group, which had a positive trend. The incidence of adenoma (3/50) in the 100 mg/m^3 group, two of which consisted of multiple adenomas, exceeded the historical control incidences for inhalation studies (0/150) and all routes (0/300); however, the response was not robust and the incidence was not significantly increased compared to the chamber controls. As in male rats, the incidences of alveolar epithelium hyperplasia and alveolar/bronchiolar epithelium hyperplasia were significantly increased ($P<0.001$) in all exposed groups of female rats. The severities of these nonneoplastic lesions increased from minimal to mild with increasing exposure concentration. However, there were no carcinomas observed in female rats, while two were observed in the male rats. Taken together, the low incidences of alveolar/bronchiolar adenoma were considered to be equivocal evidence of carcinogenic activity in female rats.

Additional findings in the rats included a cystic keratinizing epithelioma in one female exposed to 100 mg/m^3 and several other nonneoplastic lesions which increased in severity and incidence with increasing exposure concentration. Alveolar epithelium hyperplasia and alveolar/bronchiolar epithelium hyperplasia, fibrosis, histiocytic cellular infiltration, and chronic active inflammation were significantly increased in all exposed groups of males and females. There were also significantly increased incidences of alveolar epithelium squamous metaplasia, lymphohistiocytic hyperplasia of the bronchus-associated lymphoid tissue, and alveolar proteinosis in some exposed groups of males and females. These nonneoplastic lesions are commonly observed in inhalation studies of irritant gases, vapors, and particulates.

In male and female mice exposed to TRIM VX for 2 years, there was clear evidence of carcinogenic activity in the lung. In males, this was based on the significantly increased incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in the 100 mg/m^3 group, which occurred with a positive trend. Also, the combined incidence in the 100 mg/m^3 group (46%) exceeded the historical control ranges for inhalation studies (26% to 32%) and all routes (16% to 38%). In males, the incidence of multiple alveolar/bronchiolar carcinoma was significantly increased in the 100 mg/m^3 group. In females, the incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) were significantly increased in the 100 mg/m^3 group, and there were positive trends in the incidences of these neoplasms. In 100 mg/m^3 females, the incidence of alveolar/ bronchiolar carcinoma (28%) and the combined incidence of alveolar/bronchiolar adenoma or carcinoma (40%) exceeded the historical control ranges for inhalation studies (2% to 10% and 6% to 18%, respectively). There were increased incidences of multiple alveolar/bronchiolar adenoma and multiple alveolar/bronchiolar carcinoma in females, but the incidences were not statistically significant. The incidences of alveolar epithelium hyperplasia and alveolar/bronchiolar epithelium hyperplasia were significantly increased ($P<0.001$) in both male and female mice exposed to 100 mg/m^3. In general, these lesions were qualitatively similar to those observed in rats. As noted for rats, the severities of these lesions increased with increasing exposure concentration and may have contributed to the development of adenoma and carcinoma in the lung.

Additional nonneoplastic lung lesions noted in male and female mice after a 2-year exposure to TRIM VX included histiocytic cellular infiltration and chronic inflammation. There were also significantly increased incidences of lung fibrosis in both sexes. These lesions were qualitatively similar to the nonneoplastic lung lesions observed in rats but were not as severe. In general, the incidences increased with increasing exposure concentration and were most severe in the 100 mg/m^3 groups.

In the 2-year studies, lung neoplasms were accompanied by a range of inflammatory and nonneoplastic lesions of the respiratory tract. Prolonged exposure to TRIM VX appeared to cause progressive injury to the lungs in both rats and mice, as indicated by the increased incidences and severities of the nonneoplastic lesions relative to the 3-month studies, and may have contributed to the development of pulmonary neoplasms in mice. There have been no epidemiologic studies evaluating the potential carcinogenicity of TRIM VX exposure. The epidemiologic data that are available do not show an association between lung cancer and exposure to other soluble oil metalworking fluids (Calvert *et al.*, 1998; Eisen *et al.*, 2001; Friesen *et al.*, 2011). However, most retrospective evaluations that associate metalworking fluid exposure and cancer in humans are limited by the facts that these products are chemical mixtures that change over time, exposure information was not always attributed to a particular class of metalworking fluid, and confounding personal behaviors such as smoking were also present.

Several other nonneoplastic lesions were noted in the nose, larynx, and lymph nodes of both rats and mice following TRIM VX exposure for 2 years; however, there was no indication of carcinogenicity at these sites. In the nasal cavity of rats, there were significantly increased incidences of olfactory epithelium glands hyperplasia, goblet cell hyperplasia, suppurative inflammation, and olfactory and respiratory epithelium hyaline droplet accumulation in all exposed groups of males and females. There were also significantly increased incidences of respiratory epithelium hyperplasia in some exposed groups of males and females and transitional epithelium hyperplasia in females. In the nasal cavity of all exposed groups of male and female mice, there were significantly increased incidences of exudate, chronic active inflammation, and olfactory and respiratory epithelium hyaline droplet accumulation. In addition, there were significantly increased incidences of atrophy and necrosis in the respiratory epithelium of some groups of male and female mice. The incidences of turbinate atrophy, turbinate perforation, and nasopharyngeal duct perforation were increased in male and female mice exposed to 30 or 100 mg/m^3. With the exception of exudate and associated turbinate and nasopharyngeal duct perforation, these lesions are not uncommon in inhalation studies with irritating gases, aerosols, or particulates. An increased incidence of nasal carcinoma has not been reported in metalworking fluid workers.

In the larynx of rats, there were significantly increased incidences of squamous hyperplasia and squamous metaplasia of the epiglottis and mixed cell infiltration cellular in all exposed male and female groups. Similarly, in mice there were significantly increased incidences of squamous hyperplasia of the epiglottis in 30 and 100 mg/m^3 males and females and squamous metaplasia of the epiglottis in all exposed groups of males and females. These lesions in the larynx were morphologically similar in rats and mice. In inhalation studies with laboratory rodents, squamous hyperplasia and squamous metaplasia are considered adaptive/protective responses to chronic irritation or injury to the laryngeal epithelium, rather than preneoplastic responses (Renne and Gideon, 2006). These lesions are not considered preneoplastic in the larynx since they have not been accompanied by laryngeal squamous cell tumors in rats or mice in NTP studies. Although cancer of the larynx is rare in rodents, squamous cell carcinoma of the larynx is one of the most common cancers of the respiratory tract in humans (Heroiu Cataloiu *et al*., 2013). Epidemiologic studies have reported increased laryngeal cancer in workers exposed to straight oil metalworking fluids (Eisen *et al*., 1994); however, the evidence was significantly less for workers exposed to soluble oils (Tolbert *et al*., 1992; Friesen *et al*., 2011).

Nonneoplastic lesions were also noted in the lymph nodes of rats and mice. In the bronchial and mediastinal lymph nodes of rats, there were significantly increased incidences of lymphohistiocytic hyperplasia in all exposed groups of males and females and the severities increased with exposure concentration. In the bronchial lymph node of male mice, there were significantly increased incidences of lymphoid hyperplasia and histiocytic cellular infiltration that were morphologically similar to the nonneoplastic lesions that occurred in the lymph nodes of male and female rats and diagnosed as lymphohistiocytic hyperplasia. These lymph nodes drain the lung, thus the changes observed most likely reflect the inflammatory lesions in the lung.

There are no published genotoxicity data for TRIM VX. In the current study, TRIM VX was not mutagenic in several bacterial strains and no indication of chromosomal damage in erythrocytes of rats or mice was observed after 3 months of exposure to TRIM VX. The NTP has evaluated the mutagenicity of a variety of different metalworking fluids in bacteria and most were shown to be negative (Waters *et al*., 2008). However, older formulations of metalworking fluids that contained nitrosating agents were associated with DNA damage in a single epidemiologic study (Fuchs *et al*., 1995). Although newer formulations of metalworking fluids used after 1980 do not contain nitrosating agents, it is possible that other components of these mixtures may have genotoxic potential. To that end, several components of TRIM VX have been tested for mutagenicity and all but one were found to be inactive. The one exception is α-terpineol, which demonstrated weak activity in *Salmonella typhimurium* strain TA102, a strain that is sensitive to oxygen radicals (Gomes-Carneiro *et al*., 1998).

Overall, a wide spectrum of nonneoplastic lesions of the lung, nose, larynx, and lymph nodes were significantly increased across exposure groups, many of which were observed at the lowest concentration tested (10 mg/m^3). Workers exposed to metalworking fluids report a wide variety of respiratory conditions including hypersensitivity pneumonitis, impaired lung function, and asthma. Furthermore, work-related asthma is one of the most prevalent occupational disorders and is associated with significant costs in healthcare and workers' compensation (NIOSH, 2013b). Although there has been no previous association between metalworking fluid exposure and lung cancer, this study also suggests that chronic exposure to TRIM VX may lead to the development of lung tumors. Interestingly, evidence of systemic toxicity or carcinogenicity was minimal in animals exposed to TRIM VX, which implies that TRIM VX-related toxicity may be limited to the site of contact.

TRIM VX toxicity was evaluated as part of a larger comparative assessment of currently marketed metalworking fluids. In this paradigm, TRIM VX results can be compared to results previously obtained in a NTP carcinogenicity study of CIMSTAR 3800, a semisynthetic metalworking fluid (NTP, 2015). In both 2-year studies, identical exposure concentrations (10, 30, and 100 mg/m^3) were evaluated by inhalation in B6C3F1/N mice and Wistar Han rats. Overall, rats and mice in the TRIM VX studies had a greater spectrum of inflammatory and nonneoplastic proliferative lesions in the lung, nose, and larynx when compared to animals exposed to CIMSTAR 3800. One notable difference between the two metalworking fluids was the occurrence of fibrosis in the lungs of rats and mice in TRIM VX studies. Fibrosis was not observed in the CIMSTAR 3800 studies. In rats and mice, pulmonary fibrosis is a common response to particulate exposure and is usually associated with areas of chronic injury and inflammation (NTP, 1998, 2001, 2002). Fibrosis is not considered to be due to direct injury to the fibroblast but is secondary to the chronic inflammatory processes in the lungs of exposed rodents and related to production and release of potentially fibrogenic mediators, fibronectin, and growth factors by alveolar macrophages and other inflammatory cells (Renne *et al*., 2003; Harkema *et al*., 2013). The lack of fibrosis in the CIMSTAR 3800 studies may be entirely due to the lack of significant inflammatory responses in the lungs of rats and mice compared to that observed in rats and mice exposed to TRIM VX.

Furthermore, there was clear evidence for carcinogenicity in male and female mice exposed to TRIM VX and only some evidence for carcinogenicity in female mice exposed to CIMSTAR 3800 based on the incidence of lung tumors. In the rats exposed to TRIM VX, lung tumors may have been treatment related but there was no evidence for carcinogenicity in rats exposed to CIMSTAR 3800 for 2 years. There was evidence of systemic toxicity in mice exposed to CIMSTAR 3800 based on an increase in the incidences of nonneoplastic lesions and follicular cell carcinoma in the thyroid gland of females. Furthermore, there was equivocal evidence for carcinogenic activity in the prostate gland, brain, skin, and uterus of rats exposed to CIMSTAR 3800 for 2 years. In contrast, evidence for systemic toxicity or carcinogenic activity was minimal in rodents exposed to TRIM VX. To summarize, these data suggest that TRIM VX toxicity may be limited to the site of contact whereas CIMSTAR 3800 exposure may elicit a broader range of toxicity (NTP, 2015).

In previous literature, metalworking fluid toxicity was speculated to be associated with chemical by-products, accumulating metals, added biocides or anticorrosives, bacterial growth, and endotoxins (Gordon and Galdanes, 1999; Weiss *et al*., 2002; Lim *et al*., 2005a,b; Monteiro-Riviere *et al*., 2006). In the TRIM VX and CIMSTAR 3800 studies, only unused formulations were evaluated in order to eliminate the confounding effects of contaminants. Although a complete chemical analysis was not performed, several major chemical groups were identified in TRIM VX (soluble oil) and CIMSTAR 3800 (semisynthetic). Notable differences in water and oil content, and oil-soluble components (Table H2), could account for the variances in the nonneoplastic lung responses that occurred in the TRIM VX and CIMSTAR 3800 studies.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *equivocal evidence of carcinogenic activity** of TRIM VX in male Wistar Han rats based on the combined occurrences of alveolar/bronchiolar adenoma or carcinoma of the lung. There was *equivocal evidence of carcinogenic activity* of TRIM VX in female Wistar Han rats based on the occurrences of alveolar/bronchiolar adenoma of the lung. There was *clear evidence of carcinogenic activity* of TRIM VX in male B6C3F1/N mice based on the increased combined incidences of alveolar/bronchiolar adenoma or carcinoma of the lung. There was *clear evidence of carcinogenic activity* of TRIM VX in female B6C3F1/N mice based on the increased combined incidences of alveolar/bronchiolar adenoma or carcinoma (primarily carcinoma) of the lung.

Exposure to TRIM VX resulted in increased incidences of nonneoplastic lesions of the lung, nose, and larynx in male and female rats and mice, the bronchial lymph node in male and female rats and male mice, and the mediastinal lymph node in male and female rats.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Peer Review Panel comments and the public discussion on this Technical Report appears in Appendix K.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR INHALATION STUDY OF TRIM VX

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TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of TRIM VX[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	12	10	14	15
Natural deaths	2	1	3	1
Survivors				
Terminal kill	36	39	33	34
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	1 (2%)	1 (2%)		1 (2%)
Mesentery	(2)	(2)	(3)	(5)
Pancreas	(50)	(50)	(50)	(50)
Acinus, adenoma	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Schwannoma malignant				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma				1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Schwannoma malignant, metastatic, skeletal muscle			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Carcinoma				2 (4%)
Adrenal medulla	(50)	(50)	(49)	(49)
Pheochromocytoma benign		2 (4%)	1 (2%)	
Pheochromocytoma malignant				1 (2%)
Schwannoma malignant, metastatic, skeletal muscle			1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	5 (10%)	1 (2%)		1 (2%)
Carcinoma				1 (2%)
Parathyroid gland	(39)	(44)	(46)	(49)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	13 (26%)	12 (24%)	14 (28%)	15 (30%)
Pars distalis, adenoma, multiple	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Pars intermedia, adenoma	1 (2%)	1 (2%)	2 (4%)	
Pars nervosa, granular cell tumor benign			1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m³	30 mg/m³	100 mg/m³
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, c-cell, adenoma				1 (2%)
C-cell, adenoma	3 (6%)	5 (10%)	2 (4%)	6 (12%)
Follicle, adenoma		1 (2%)	3 (6%)	1 (2%)
Follicle, carcinoma		1 (2%)	1 (2%)	
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(49)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Interstitial cell, adenoma		1 (2%)	2 (4%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(11)	(7)	(7)	(8)
Lumbar, hemangioma	1 (9%)			
Lymph node, bronchial	(39)	(43)	(42)	(42)
Lymph node, mandibular	(46)	(46)	(46)	(48)
Lymph node, mediastinal	(43)	(48)	(44)	(43)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)	1 (2%)	
Spleen	(50)	(50)	(50)	(50)
Thymus	(48)	(49)	(50)	(50)
Schwannoma malignant, metastatic, skeletal muscle			1 (2%)	
Thymoma benign	3 (6%)	3 (6%)	4 (8%)	1 (2%)
Integumentary System				
Mammary gland	(4)	(5)	(1)	(2)
Skin	(50)	(50)	(50)	(50)
Epidermis, keratoacanthoma	1 (2%)	1 (2%)		1 (2%)
Epidermis, squamous cell papilloma		2 (4%)		
Sebaceous gland, adenoma	1 (2%)			
Subcutaneous tissue, fibroma	1 (2%)	1 (2%)	1 (2%)	
Subcutaneous tissue, hemangiosarcoma		1 (2%)		
Subcutaneous tissue, schwannoma malignant		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(4)	(4)	(8)	(3)
Hemangiosarcoma				2 (67%)
Rhabdomyosarcoma		1 (25%)		
Schwannoma malignant			1 (13%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, Zymbal's gland	1 (2%)			
Granular cell tumor benign		1 (2%)		
Oligodendroglioma benign			1 (2%)	
Peripheral nerve	(4)	(2)	(8)	(2)
Schwannoma malignant			1 (13%)	
Spinal cord	(4)	(2)	(7)	(2)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma				1 (2%)
Alveolar/bronchiolar carcinoma				2 (4%)
Carcinoma, metastatic, Zymbal's gland	1 (2%)			
Schwannoma malignant, metastatic, skeletal muscle			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Polyp, multiple				1 (2%)
Squamous cell papilloma				1 (2%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Lacrimal gland	(1)	(0)	(0)	(1)
Zymbal's gland	(1)	(0)	(0)	(0)
Carcinoma	1 (100%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, carcinoma	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma				1 (2%)
Lymphoma malignant	1 (2%)	1 (2%)		1 (2%)
Mesothelioma malignant	1 (2%)	1 (2%)		
Mesothelioma NOS	1 (2%)		1 (2%)	1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Neoplasm Summary				
Total animals with primary neoplasms[c]	26	27	24	31
Total primary neoplasms	38	40	37	47
Total animals with benign neoplasms	24	25	23	27
Total benign neoplasms	33	33	32	34
Total animals with malignant neoplasms	4	6	3	10
Total malignant neoplasms	4	7	4	12
Total animals with metastatic neoplasms	1		1	
Total metastatic neoplasms	2		4	
Total animals with uncertain neoplasms-benign or malignant	1		1	1
Total uncertain neoplasms	2		1	2

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate[a]	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate[b]	0.0%	0.0%	0.0%	7.2%
Terminal rate[c]	0/36 (0%)	0/39 (0%)	0/33 (0%)	3/34 (9%)
First incidence (days)	—[e]	—	—	729 (T)
Poly-3 test[d]	P=0.007	—[f]	—	P=0.106
Pancreatic Islets: Adenoma				
Overall rate	5/50 (10%)	1/50 (2%)	0/50 (0%)	1/50 (2%)
Adjusted rate	11.1%	2.1%	0.0%	2.4%
Terminal rate	3/36 (8%)	0/39 (0%)	0/33 (0%)	0/34 (0%)
First incidence (days)	662	715	—	540
Poly-3 test	P=0.172N	P=0.092N	P=0.037N	P=0.117N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	1/50 (2%)	0/50 (0%)	2/50 (4%)
Adjusted rate	11.1%	2.1%	0.0%	4.7%
Terminal rate	3/36 (8%)	0/39 (0%)	0/33 (0%)	1/34 (3%)
First incidence (days)	662	715	—	540
Poly-3 test	P=0.394N	P=0.092N	P=0.037N	P=0.244N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	14/50 (28%)	13/50 (26%)	15/50 (30%)	16/50 (32%)
Adjusted rate	29.6%	27.3%	33.0%	34.0%
Terminal rate	7/36 (19%)	9/39 (23%)	6/33 (18%)	7/34 (21%)
First incidence (days)	421	684	452	367
Poly-3 test	P=0.310	P=0.492N	P=0.447	P=0.403
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	2.2%	6.3%	0.0%	2.4%
Terminal rate	1/36 (3%)	2/39 (5%)	0/33 (0%)	1/34 (3%)
First incidence (days)	729 (T)	323	—	729 (T)
Poly-3 test	P=0.462N	P=0.331	P=0.513N	P=0.744
Thymus: Benign Thymoma				
Overall rate	3/48 (6%)	3/49 (6%)	4/50 (8%)	1/50 (2%)
Adjusted rate	6.9%	6.3%	9.5%	2.4%
Terminal rate	2/34 (6%)	2/39 (5%)	3/33 (9%)	1/34 (3%)
First incidence (days)	535	589	666	729 (T)
Poly-3 test	P=0.252N	P=0.619N	P=0.485	P=0.320N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	3/50 (6%)	5/50 (10%)	2/50 (4%)	7/50 (14%)
Adjusted rate	6.7%	10.6%	4.8%	16.3%
Terminal rate	3/36 (8%)	5/39 (13%)	2/33 (6%)	4/34 (12%)
First incidence (days)	729 (T)	729 (T)	729 (T)	409
Poly-3 test	P=0.105	P=0.385	P=0.529N	P=0.140
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	0.0%	2.1%	7.2%	2.4%
Terminal rate	0/36 (0%)	1/39 (3%)	3/33 (9%)	1/34 (3%)
First incidence (days)	—	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.489	P=0.510	P=0.107	P=0.486

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	0/50 (0%)	2/50 (4%)	4/50 (8%)	1/50 (2%)
Adjusted rate	0.0%	4.3%	9.4%	2.4%
Terminal rate	0/36 (0%)	2/39 (5%)	3/33 (9%)	1/34 (3%)
First incidence (days)	—	729 (T)	551	729 (T)
Poly-3 test	P=0.595	P=0.249	P=0.054	P=0.486
All Organs: Hemangiosarcoma				
Overall rate	0/50 (0%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	4.3%	2.4%	7.0%
Terminal rate	0/36 (0%)	2/39 (5%)	1/33 (3%)	1/34 (3%)
First incidence (days)	—	729 (T)	729 (T)	459
Poly-3 test	P=0.122	P=0.249	P=0.487	P=0.111
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	1/50 (2%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	2.2%	4.3%	2.4%	7.0%
Terminal rate	0/36 (0%)	2/39 (5%)	1/33 (3%)	1/34 (3%)
First incidence (days)	626	729 (T)	729 (T)	459
Poly-3 test	P=0.227	P=0.515	P=0.744	P=0.288
All Organs: Benign Neoplasms				
Overall rate	24/50 (48%)	25/50 (50%)	23/50 (46%)	27/50 (54%)
Adjusted rate	49.7%	50.7%	49.6%	57.3%
Terminal rate	15/36 (42%)	17/39 (44%)	13/33 (39%)	17/34 (50%)
First incidence (days)	421	323	270	367
Poly-3 test	P=0.243	P=0.540	P=0.578N	P=0.293
All Organs: Malignant Neoplasms				
Overall rate	4/50 (8%)	6/50 (12%)	3/50 (6%)	10/50 (20%)
Adjusted rate	8.8%	12.4%	7.0%	22.9%
Terminal rate	2/36 (6%)	4/39 (10%)	1/33 (3%)	7/34 (21%)
First incidence (days)	421	323	551	459
Poly-3 test	P=0.029	P=0.408	P=0.538N	P=0.059
All Organs: Benign or Malignant Neoplasms				
Overall rate	26/50 (52%)	27/50 (54%)	24/50 (48%)	31/50 (62%)
Adjusted rate	53.8%	54.2%	51.1%	63.7%
Terminal rate	17/36 (47%)	18/39 (46%)	13/33 (39%)	19/34 (56%)
First incidence (days)	421	323	270	367
Poly-3 test	P=0.149	P=0.566	P=0.476N	P=0.214

(T) Terminal kill

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for lung, pancreatic islets, pituitary gland, thymus, and thyroid gland; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE A3
Historical Incidence of Lung Neoplasms in Control Male Wistar Han Rats[a]

Study (Study Start)	Alveolar/bronchiolar Adenoma	Alveolar/bronchiolar Carcinoma	Alveolar/bronchiolar Adenoma or Carcinoma
Historical Incidence: Inhalation Studies			
Antimony trioxide (September 2008)	3/50	0/50	3/50
CIMSTAR 3800 (April 2008)	1/50	0/50	1/50
TRIM VX (July 2009)	0/50	0/50	0/50
Total (%)	4/150 (2.7%)	0/150	4/150 (2.7%)
Mean ± standard deviation	2.7% ± 3.1%		2.7% ± 3.1%
Range	0%-6%		0%-6%
Overall Historical Incidence: All Routes			
Total (%)	4/299 (1.3%)	0/299	4/299 (1.3%)
Mean ± standard deviation	1.3% ± 2.4%		1.3% ± 2.4%
Range	0%-6%		0%-6%

[a] Data as of November 2014

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of TRIM VX[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	12	10	14	15
Natural deaths	2	1	3	1
Survivors				
Terminal kill	36	39	33	34
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			1 (2%)
Artery, inflammation	1 (2%)			
Lymphoid tissue, hyperplasia				1 (2%)
Intestine large, colon	(50)	(50)	(50)	(50)
Lymphoid tissue, hyperplasia			1 (2%)	
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Artery, inflammation	1 (2%)			
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis	5 (10%)	2 (4%)		4 (8%)
Basophilic focus	5 (10%)	10 (20%)	15 (30%)	9 (18%)
Clear cell focus	14 (28%)	23 (46%)	17 (34%)	23 (46%)
Eosinophilic focus	2 (4%)			2 (4%)
Fatty change			1 (2%)	1 (2%)
Hepatodiaphragmatic nodule	1 (2%)	2 (4%)		1 (2%)
Infiltration cellular, lymphocyte		1 (2%)		
Inflammation, granulomatous		1 (2%)		2 (4%)
Mixed cell focus		1 (2%)	2 (4%)	1 (2%)
Bile duct, cyst			2 (4%)	1 (2%)
Bile duct, dilatation	1 (2%)			2 (4%)
Bile duct, hyperplasia	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Serosa, fibrosis			1 (2%)	
Mesentery	(2)	(2)	(3)	(5)
Fat, hemorrhage			1 (33%)	
Fat, necrosis	2 (100%)	2 (100%)	2 (67%)	4 (80%)
Pancreas	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	1 (2%)		
Acinus, atrophy	7 (14%)	2 (4%)	9 (18%)	2 (4%)
Acinus, hyperplasia			1 (2%)	
Acinus, inflammation	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Inflammation	1 (2%)			
Stomach, glandular	(50)	(50)	(50)	(50)
Mineralization				1 (2%)
Artery, inflammation	1 (2%)			

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	21 (42%)	17 (34%)	22 (44%)	18 (36%)
Endocardium, hyperplasia			2 (4%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	4 (8%)	1 (2%)	1 (2%)	3 (6%)
Hypertrophy	13 (26%)	18 (36%)	13 (26%)	15 (30%)
Necrosis	2 (4%)			
Thrombosis			1 (2%)	
Vacuolization cytoplasmic	19 (38%)	23 (46%)	21 (42%)	21 (42%)
Adrenal medulla	(50)	(50)	(49)	(49)
Hyperplasia	1 (2%)	1 (2%)		1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Parathyroid gland	(39)	(44)	(46)	(49)
Fibrosis	1 (3%)			
Hyperplasia		1 (2%)	1 (2%)	
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Cyst	2 (4%)	2 (4%)	4 (8%)	1 (2%)
Pars distalis, hyperplasia	12 (24%)	15 (30%)	8 (16%)	11 (22%)
Pars intermedia, hyperplasia			3 (6%)	
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	36 (72%)	39 (78%)	34 (68%)	40 (80%)
Follicle, hyperplasia	4 (8%)	7 (14%)	4 (8%)	1 (2%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm		1 (2%)		
Preputial gland	(49)	(50)	(50)	(50)
Cyst	1 (2%)			
Fibrosis				1 (2%)
Inflammation	17 (35%)	13 (26%)	11 (22%)	16 (32%)
Prostate	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		
Fibrosis		1 (2%)		
Inflammation	17 (34%)	12 (24%)	10 (20%)	10 (20%)
Seminal vesicle	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Fibrosis	1 (2%)			
Inflammation	4 (8%)		1 (2%)	
Testes	(50)	(50)	(50)	(50)
Cyst				1 (2%)
Edema	3 (6%)	2 (4%)	1 (2%)	2 (4%)
Artery, inflammation, chronic	3 (6%)			1 (2%)
Germinal epithelium, atrophy	3 (6%)	8 (16%)		6 (12%)
Germinal epithelium, hypoplasia			1 (2%)	
Interstitial cell, hyperplasia	2 (4%)			1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m³	30 mg/m³	100 mg/m³
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(11)	(7)	(7)	(8)
Axillary, hyperplasia, plasma cell		1 (14%)		
Iliac, hyperplasia, plasma cell	1 (9%)	2 (29%)	4 (57%)	1 (13%)
Lumbar, hyperplasia, lymphoid				1 (13%)
Lumbar, hyperplasia, plasma cell	7 (64%)	5 (71%)	3 (43%)	4 (50%)
Renal, hyperplasia, plasma cell	2 (18%)			2 (25%)
Lymph node, bronchial	(39)	(43)	(42)	(42)
Hyperplasia, lymphohistiocytic		17 (40%)	29 (69%)	35 (83%)
Lymph node, mandibular	(46)	(46)	(46)	(48)
Hyperplasia, plasma cell	1 (2%)	1 (2%)		
Lymph node, mediastinal	(43)	(48)	(44)	(43)
Congestion				1 (2%)
Hyperplasia, lymphohistiocytic		20 (42%)	22 (50%)	32 (74%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Hyperplasia, lymphohistiocytic				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Congestion			1 (2%)	
Cyst		1 (2%)		
Hematopoietic cell proliferation	7 (14%)	9 (18%)	8 (16%)	8 (16%)
Hemorrhage		1 (2%)		
Lymphoid follicle, atrophy	3 (6%)		2 (4%)	
Thymus	(48)	(49)	(50)	(50)
Atrophy	35 (73%)	36 (73%)	26 (52%)	26 (52%)
Cyst			1 (2%)	
Integumentary System				
Mammary gland	(4)	(5)	(1)	(2)
Galactocele	1 (25%)			
Hyperplasia	1 (25%)			
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Hyperkeratosis	1 (2%)			
Inflammation	2 (4%)	1 (2%)		4 (8%)
Ulcer	16 (32%)	14 (28%)	13 (26%)	13 (26%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis				1 (2%)
Skeletal muscle	(4)	(4)	(8)	(3)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	8 (16%)	6 (12%)	8 (16%)	7 (14%)
Degeneration	1 (2%)			
Gliosis	1 (2%)			
Hemorrhage	1 (2%)			
Hydrocephalus	5 (10%)	3 (6%)	8 (16%)	4 (8%)
Necrosis			1 (2%)	
Meninges, inflammation	1 (2%)			
Peripheral nerve	(4)	(2)	(8)	(2)
Axon, degeneration			2 (25%)	
Spinal cord	(4)	(2)	(7)	(2)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	$10\ mg/m^3$	$30\ mg/m^3$	$100\ mg/m^3$
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Foreign body	1 (2%)		1 (2%)	
Infiltration cellular, mixed cell	1 (2%)	9 (18%)	27 (54%)	31 (62%)
Inflammation, chronic active	1 (2%)	1 (2%)		
Epiglottis, hyperplasia, squamous		26 (52%)	48 (96%)	50 (100%)
Epiglottis, metaplasia, squamous	3 (6%)	50 (100%)	50 (100%)	50 (100%)
Lung	(50)	(50)	(50)	(50)
Fibrosis	4 (8%)	43 (86%)	45 (90%)	49 (98%)
Foreign body			1 (2%)	
Hyperplasia, lymphohistiocytic		1 (2%)	1 (2%)	
Infiltration cellular, histiocyte	14 (28%)	50 (100%)	50 (100%)	50 (100%)
Inflammation, chronic active	7 (14%)	46 (92%)	46 (92%)	48 (96%)
Alveolar/bronchiolar epithelium, hyperplasia	4 (8%)	22 (44%)	39 (78%)	46 (92%)
Alveolar epithelium, hyperplasia	11 (22%)	43 (86%)	45 (90%)	49 (98%)
Alveolar epithelium, metaplasia, squamous				5 (10%)
Alveolus, proteinosis		1 (2%)	31 (62%)	45 (90%)
Bronchus-associated lymphoid tissue, hyperplasia, lymphohistiocytic		1 (2%)	4 (8%)	6 (12%)
Mediastinum, inflammation, chronic		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Foreign body	1 (2%)		1 (2%)	1 (2%)
Inflammation, suppurative	7 (14%)	46 (92%)	47 (94%)	46 (92%)
Glands, olfactory epithelium, hyperplasia		43 (86%)	41 (82%)	49 (98%)
Goblet cell, hyperplasia	5 (10%)	36 (72%)	37 (74%)	42 (84%)
Goblet cell, nasolacrimal duct, metaplasia		1 (2%)		
Goblet cell, olfactory epithelium, hyperplasia			1 (2%)	
Nasolacrimal duct, inflammation, chronic		1 (2%)		
Nasolacrimal duct, metaplasia, squamous		1 (2%)		
Olfactory epithelium, accumulation, hyaline droplet	20 (40%)	50 (100%)	50 (100%)	50 (100%)
Olfactory epithelium, atrophy		1 (2%)	1 (2%)	
Olfactory epithelium, metaplasia, squamous		1 (2%)		1 (2%)
Respiratory epithelium, accumulation, hyaline droplet	10 (20%)	50 (100%)	48 (96%)	50 (100%)
Respiratory epithelium, hyperplasia	2 (4%)	7 (14%)	7 (14%)	19 (38%)
Transitional epithelium, hyperplasia	7 (14%)	7 (14%)	8 (16%)	13 (26%)
Transitional epithelium, metaplasia, squamous	2 (4%)	2 (4%)	3 (6%)	6 (12%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract			1 (2%)	1 (2%)
Inflammation			1 (2%)	
Retina, atrophy	5 (10%)	5 (10%)	1 (2%)	3 (6%)
Harderian gland	(50)	(50)	(50)	(50)
Cyst		1 (2%)		
Hyperplasia	1 (2%)	1 (2%)		1 (2%)
Inflammation	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Lacrimal gland	(1)	(0)	(0)	(1)
Atrophy	1 (100%)			1 (100%)
Zymbal's gland	(1)	(0)	(0)	(0)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst			1 (2%)	
Hydronephrosis	1 (2%)		1 (2%)	3 (6%)
Infarct		1 (2%)	2 (4%)	
Inflammation, granulomatous				1 (2%)
Metaplasia, lipocyte			1 (2%)	1 (2%)
Necrosis		1 (2%)		
Nephropathy	27 (54%)	31 (62%)	27 (54%)	28 (56%)
Pelvis, inflammation	19 (38%)	17 (34%)	5 (10%)	9 (18%)
Renal tubule, cyst		1 (2%)	1 (2%)	
Renal tubule, hyperplasia			1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Inflammation		1 (2%)	1 (2%)	
Transitional epithelium, hyperplasia			1 (2%)	

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF TRIM VX

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TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of TRIM VX[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	19	15	15	16
Natural deaths	1	2	2	4
Survivors				
Died last week of study	1	1		
Terminal kill	29	32	33	30
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Neoplasm NOS		1 (2%)		
Intestine large, colon	(50)	(50)	(50)	(50)
Neoplasm NOS		1 (2%)		
Schwannoma malignant, metastatic, uterus			1 (2%)	
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Cholangioma		1 (2%)		
Hepatocellular adenoma				1 (2%)
Mesentery	(4)	(5)	(9)	(4)
Adenocarcinoma, metastatic, uterus			1 (11%)	
Neoplasm NOS		1 (20%)		
Schwannoma malignant, metastatic, uterus			1 (11%)	
Pancreas	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus			1 (2%)	
Neoplasm NOS		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Neoplasm NOS		1 (2%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Neoplasm NOS		1 (2%)		
Tongue	(0)	(0)	(0)	(1)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Schwannoma malignant				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma				2 (4%)
Adrenal medulla	(50)	(50)	(50)	(49)
Pheochromocytoma malignant			1 (2%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Parathyroid gland	(42)	(43)	(47)	(47)
Adenoma	1 (2%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Endocrine System (continued)				
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	34 (68%)	31 (62%)	26 (52%)	22 (44%)
Pars distalis, adenoma, multiple		2 (4%)	6 (12%)	2 (4%)
Pars intermedia, adenoma	1 (2%)		1 (2%)	
Thyroid gland	(49)	(50)	(50)	(50)
Bilateral, c-cell, adenoma	1 (2%)	1 (2%)		
C-cell, adenoma	6 (12%)	3 (6%)	4 (8%)	6 (12%)
Follicle, adenoma		1 (2%)	2 (4%)	1 (2%)
Follicle, carcinoma	1 (2%)	2 (4%)		
General Body System				
None				
Genital System				
Clitoral gland	(50)	(48)	(49)	(50)
Squamous cell papilloma		1 (2%)		
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor benign		2 (4%)		2 (4%)
Granulosa cell tumor malignant	1 (2%)	1 (2%)		
Tubulostromal adenoma				1 (2%)
Tubulostromal carcinoma				1 (2%)
Bilateral, tubulostromal adenoma				1 (2%)
Uterus	(50)	(50)	(50)	(50)
Adenocarcinoma	1 (2%)	1 (2%)	4 (8%)	1 (2%)
Adenoma	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Granular cell tumor benign	1 (2%)			
Polyp stromal	4 (8%)		7 (14%)	1 (2%)
Polyp stromal, multiple	2 (4%)		1 (2%)	
Sarcoma stromal	1 (2%)		1 (2%)	
Schwannoma malignant	2 (4%)		4 (8%)	1 (2%)
Vagina	(1)	(0)	(0)	(0)
Fibrosarcoma	1 (100%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Neoplasm NOS		1 (2%)		
Lymph node	(4)	(1)	(2)	(1)
Lumbar, hemangiosarcoma		1 (100%)		
Pancreatic, adenocarcinoma, metastatic, uterus			1 (50%)	
Renal, schwannoma malignant, metastatic, uterus			1 (50%)	
Lymph node, bronchial	(37)	(37)	(44)	(36)
Lymph node, mandibular	(40)	(46)	(47)	(50)
Lymph node, mediastinal	(46)	(49)	(46)	(45)
Neoplasm NOS		1 (2%)		
Schwannoma malignant, metastatic, lung				1 (2%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Neoplasm NOS		1 (2%)		
Spleen	(50)	(50)	(50)	(50)
Thymus	(48)	(49)	(49)	(49)
Schwannoma malignant, metastatic, lung				1 (2%)
Thymoma benign	8 (17%)	4 (8%)	7 (14%)	10 (20%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m³	30 mg/m³	100 mg/m³
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Adenoma, multiple	1 (2%)			
Carcinoma	3 (6%)	2 (4%)	2 (4%)	4 (8%)
Carcinoma, multiple	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Fibroadenoma	2 (4%)	7 (14%)	7 (14%)	4 (8%)
Fibroadenoma, multiple		2 (4%)		1 (2%)
Skin	(50)	(50)	(50)	(50)
Epidermis, squamous cell papilloma			1 (2%)	2 (4%)
Subcutaneous tissue, lipoma		1 (2%)		
Subcutaneous tissue, neoplasm NOS		1 (2%)		
Subcutaneous tissue, schwannoma malignant				2 (4%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(4)	(3)	(3)	(4)
Adenocarcinoma, metastatic, uterus			1 (33%)	
Schwannoma malignant, metastatic, skin				1 (25%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Granular cell tumor benign			1 (2%)	
Oligodendroglioma benign			1 (2%)	
Peripheral nerve	(4)	(3)	(2)	(3)
Spinal cord	(4)	(3)	(2)	(3)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma			1 (2%)	1 (2%)
Alveolar/bronchiolar adenoma, multiple				2 (4%)
Carcinoma, metastatic, kidney		1 (2%)		
Carcinoma, metastatic, uterus			1 (2%)	
Cystic keratinizing epithelioma				1 (2%)
Neoplasm NOS		1 (2%)		
Schwannoma malignant, metastatic, uterus			1 (2%)	
Mediastinum, schwannoma malignant				1 (2%)
Nose	(50)	(50)	(49)	(50)
Polyp				1 (2%)
Schwannoma benign				1 (2%)
Schwannoma malignant, metastatic, skin				1 (2%)
Trachea	(49)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(0)	(2)	(1)	(1)
Carcinoma		1 (50%)	1 (100%)	1 (100%)
Squamous cell papilloma		1 (50%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Neoplasm NOS		1 (2%)		
Transitional epithelium, carcinoma		1 (2%)		
Ureter	(0)	(0)	(0)	(1)
Urinary bladder	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus				1 (2%)
Neoplasm NOS		1 (2%)		
Schwannoma malignant, metastatic, uterus			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	2 (4%)			1 (2%)
Lymphoma malignant	1 (2%)	1 (2%)		
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	43	44	41
Total primary neoplasms	79	84	85	82
Total animals with benign neoplasms	42	39	40	35
Total benign neoplasms	65	59	69	65
Total animals with malignant neoplasms	13	11	15	16
Total malignant neoplasms	14	12	16	17
Total animals with metastatic neoplasms		1	2	4
Total metastatic neoplasms		1	10	5
Total animals with uncertain neoplasms-benign or malignant		1		
Total uncertain neoplasms		13		

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Lung: Alveolar/bronchiolar Adenoma				
Overall rate[a]	0/50 (0%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate[b]	0.0%	0.0%	2.3%	6.8%
Terminal rate[c]	0/30 (0%)	0/33 (0%)	1/33 (3%)	3/30 (10%)
First incidence (days)	—[e]	—	731 (T)	731 (T)
Poly-3 test[d]	P=0.024	—[f]	P=0.511	P=0.127
Mammary Gland: Fibroadenoma				
Overall rate	2/50 (4%)	9/50 (18%)	7/50 (14%)	5/50 (10%)
Adjusted rate	4.8%	20.6%	15.6%	11.2%
Terminal rate	2/30 (7%)	6/33 (18%)	5/33 (15%)	2/30 (7%)
First incidence (days)	731 (T)	354	604	572
Poly-3 test	P=0.515N	P=0.029	P=0.095	P=0.243
Mammary Gland: Adenoma				
Overall rate	4/50 (8%)	1/50 (2%)	2/50 (4%)	1/50 (2%)
Adjusted rate	9.3%	2.4%	4.5%	2.3%
Terminal rate	1/30 (3%)	1/33 (3%)	1/33 (3%)	0/30 (0%)
First incidence (days)	535	731 (T)	626	593
Poly-3 test	P=0.231N	P=0.185N	P=0.322N	P=0.170N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	6/50 (12%)	10/50 (20%)	9/50 (18%)	6/50 (12%)
Adjusted rate	13.9%	22.9%	19.9%	13.3%
Terminal rate	3/30 (10%)	7/33 (21%)	6/33 (18%)	2/30 (7%)
First incidence (days)	535	354	604	572
Poly-3 test	P=0.303N	P=0.210	P=0.321	P=0.587N
Mammary Gland: Carcinoma				
Overall rate	4/50 (8%)	4/50 (8%)	5/50 (10%)	6/50 (12%)
Adjusted rate	9.5%	9.5%	11.2%	13.4%
Terminal rate	3/30 (10%)	3/33 (9%)	3/33 (9%)	2/30 (7%)
First incidence (days)	702	725	626	593
Poly-3 test	P=0.326	P=0.641N	P=0.539	P=0.408
Mammary Gland: Adenoma or Carcinoma				
Overall rate	8/50 (16%)	4/50 (8%)	6/50 (12%)	6/50 (12%)
Adjusted rate	18.5%	9.5%	13.4%	13.4%
Terminal rate	4/30 (13%)	3/33 (9%)	4/33 (12%)	2/30 (7%)
First incidence (days)	535	725	626	593
Poly-3 test	P=0.499N	P=0.186N	P=0.360N	P=0.361N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	9/50 (18%)	13/50 (26%)	12/50 (24%)	11/50 (22%)
Adjusted rate	20.8%	29.8%	26.4%	24.1%
Terminal rate	5/30 (17%)	9/33 (27%)	8/33 (24%)	4/30 (13%)
First incidence (days)	535	354	604	572
Poly-3 test	P=0.528N	P=0.236	P=0.356	P=0.451
Ovary: Benign or Malignant Granulosa Cell Tumor				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	2/50 (4%)
Adjusted rate	2.4%	7.1%	0.0%	4.6%
Terminal rate	1/30 (3%)	3/33 (9%)	0/33 (0%)	2/30 (7%)
First incidence (days)	731 (T)	731 (T)	—	731 (T)
Poly-3 test	P=0.581	P=0.307	P=0.489N	P=0.516

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Ovary: Tubulostromal Adenoma or Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	6.8%
Terminal rate	0/30 (0%)	0/33 (0%)	0/33 (0%)	3/30 (10%)
First incidence (days)	—	—	—	731 (T)
Poly-3 test	P=0.008	—	—	P=0.127
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	34/50 (68%)	33/50 (66%)	32/50 (64%)	24/50 (48%)
Adjusted rate	72.1%	69.7%	67.1%	49.8%
Terminal rate	20/30 (67%)	20/33 (61%)	21/33 (64%)	11/30 (37%)
First incidence (days)	534	346	516	436
Poly-3 test	P=0.007N	P=0.489N	P=0.377N	P=0.018N
Thymus: Benign Thymoma				
Overall rate	8/48 (17%)	4/49 (8%)	7/49 (14%)	10/49 (20%)
Adjusted rate	19.6%	9.5%	16.1%	22.7%
Terminal rate	6/28 (21%)	4/33 (12%)	6/32 (19%)	6/29 (21%)
First incidence (days)	613	731 (T)	614	436
Poly-3 test	P=0.180	P=0.157N	P=0.442N	P=0.468
Thyroid Gland (C-Cell): Adenoma				
Overall rate	7/49 (14%)	4/50 (8%)	4/50 (8%)	6/50 (12%)
Adjusted rate	16.8%	9.5%	9.1%	13.5%
Terminal rate	5/29 (17%)	4/33 (12%)	4/33 (12%)	5/30 (17%)
First incidence (days)	615	731 (T)	731 (T)	577
Poly-3 test	P=0.565	P=0.252N	P=0.227N	P=0.451N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	1/49 (2%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	2.4%	7.1%	4.5%	2.3%
Terminal rate	0/29 (0%)	3/33 (9%)	1/33 (3%)	1/30 (3%)
First incidence (days)	697	731 (T)	677	731 (T)
Poly-3 test	P=0.398N	P=0.315	P=0.527	P=0.746N
Uterus: Stromal Polyp				
Overall rate	6/50 (12%)	0/50 (0%)	8/50 (16%)	1/50 (2%)
Adjusted rate	14.2%	0.0%	18.1%	2.3
Terminal rate	5/30 (17%)	0/33 (0%)	7/33 (21%)	1/30 (3%)
First incidence (days)	618	—	708	731 (T)
Poly-3 test	P=0.130N	P=0.015N	P=0.421	P=0.049N
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	7/50 (14%)	0/50 (0%)	9/50 (18%)	1/50 (2%)
Adjusted rate	16.5%	0.0%	20.3%	2.3%
Terminal rate	5/30 (17%)	0/33 (0%)	7/33 (21%)	1/30 (3%)
First incidence (days)	618	—	689	731 (T)
Poly-3 test	P=0.089N	P=0.007N	P=0.431	P=0.026N
Uterus: Malignant Schwannoma				
Overall rate	2/50 (4%)	0/50 (0%)	4/50 (8%)	1/50 (2%)
Adjusted rate	4.7%	0.0%	8.8%	2.3%
Terminal rate	0/30 (0%)	0/33 (0%)	1/33 (3%)	1/30 (3%)
First incidence (days)	536	—	590	731 (T)
Poly-3 test	P=0.526N	P=0.240N	P=0.366	P=0.491N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Uterus: Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	4/50 (8%)	1/50 (2%)
Adjusted rate	2.4%	2.4%	9.0%	2.3%
Terminal rate	1/30 (3%)	0/33 (0%)	3/33 (9%)	0/30 (0%)
First incidence (days)	731 (T)	649	689	705
Poly-3 test	P=0.558N	P=0.758N	P=0.196	P=0.750N
Uterus: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	6/50 (12%)	2/50 (4%)
Adjusted rate	4.8%	4.7%	13.5%	4.5%
Terminal rate	2/30 (7%)	1/33 (3%)	4/33 (12%)	1/30 (3%)
First incidence (days)	731 (T)	649	677	705
Poly-3 test	P=0.519N	P=0.689N	P=0.152	P=0.676N
All Organs: Benign Neoplasms				
Overall rate	42/50 (84%)	39/50 (78%)	40/50 (80%)	35/50 (70%)
Adjusted rate	88.3%	80.9%	82.5%	71.6%
Terminal rate	27/30 (90%)	25/33 (76%)	27/33 (82%)	20/30 (67%)
First incidence (days)	534	346	516	436
Poly-3 test	P=0.031N	P=0.229N	P=0.296N	P=0.031N
All Organs: Malignant Neoplasms				
Overall rate	13/50 (26%)	11/50 (22%)	15/50 (30%)	16/50 (32%)
Adjusted rate	28.7%	25.0%	31.8%	34.9%
Terminal rate	4/30 (13%)	7/33 (21%)	7/33 (21%)	6/30 (20%)
First incidence (days)	352	104	516	593
Poly-3 test	P=0.225	P=0.438N	P=0.461	P=0.340
All Organs: Benign or Malignant Neoplasms				
Overall rate	47/50 (94%)	43/50 (86%)	44/50 (88%)	41/50 (82%)
Adjusted rate	94.0%	86.0%	88.0%	82.9%
Terminal rate	27/30 (90%)	26/33 (79%)	27/33 (82%)	23/30 (77%)
First incidence (days)	352	104	516	436
Poly-3 test	P=0.123N	P=0.159N	P=0.243N	P=0.074N

(T) Terminal kill

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for lung, ovary, pituitary gland, thymus, and thyroid gland; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE B3
Historical Incidence of Lung Neoplasms in Control Female Wistar Han Rats[a]

Study (Study Start)	Alveolar/bronchiolar Adenoma	Alveolar/bronchiolar Carcinoma	Alveolar/bronchiolar Adenoma or Carcinoma	Cystic Keratinizing Epithelioma
Historical Incidence: Inhalation Studies				
Antimony trioxide (September 2008)	0/50	0/50	0/50	0/50
CIMSTAR 3800 (April 2008)	0/50	0/50	0/50	0/50
TRIM VX (July 2009)	0/50	0/50	0/50	0/50
Total	0/150	0/150	0/150	0/150
Overall Historical Incidence: All Routes				
Total	0/300	0/300	0/300	0/300

[a] Data as of November 2014

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of TRIM VX[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	19	15	15	16
Natural deaths	1	2	2	4
Survivors				
Died last week of study	1	1		
Terminal kill	29	32	33	30
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis		4 (8%)	1 (2%)	
Basophilic focus	21 (42%)	30 (60%)	24 (48%)	25 (50%)
Clear cell focus	6 (12%)	16 (32%)	4 (8%)	8 (16%)
Eosinophilic focus	2 (4%)	3 (6%)		2 (4%)
Fatty change	2 (4%)		3 (6%)	
Fibrosis		1 (2%)		
Hematopoietic cell proliferation	1 (2%)			
Hepatodiaphragmatic nodule			1 (2%)	1 (2%)
Inflammation, granulomatous	1 (2%)	3 (6%)		2 (4%)
Mixed cell focus	3 (6%)	5 (10%)		
Necrosis			1 (2%)	
Bile duct, cyst			2 (4%)	1 (2%)
Bile duct, dilatation		1 (2%)	1 (2%)	
Bile duct, hyperplasia	2 (4%)	8 (16%)	6 (12%)	4 (8%)
Centrilobular, degeneration	1 (2%)			
Serosa, fibrosis				1 (2%)
Sinusoid, dilatation	1 (2%)			
Mesentery	(4)	(5)	(9)	(4)
Fat, necrosis	4 (100%)	4 (80%)	7 (78%)	4 (100%)
Pancreas	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)			
Acinus, atrophy	4 (8%)	2 (4%)	2 (4%)	1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Sublingual gland, inflammation		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation	2 (4%)			
Epithelium, hyperplasia	2 (4%)			
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(0)	(0)	(1)
Hyperkeratosis				1 (100%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	7 (14%)	7 (14%)	8 (16%)	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	28 (56%)	25 (50%)	28 (56%)	27 (54%)
Hematopoietic cell proliferation	3 (6%)			
Hyperplasia		1 (2%)		1 (2%)
Hypertrophy	10 (20%)	5 (10%)	15 (30%)	10 (20%)
Necrosis			2 (4%)	1 (2%)
Vacuolization cytoplasmic	9 (18%)	8 (16%)	14 (28%)	11 (22%)
Zona fasciculata, atrophy		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(49)
Hyperplasia	3 (6%)	1 (2%)		1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)			
Parathyroid gland	(42)	(43)	(47)	(47)
Fibrosis		2 (5%)	1 (2%)	
Hyperplasia	1 (2%)			
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Cyst		1 (2%)		
Pars distalis, hyperplasia	4 (8%)	13 (26%)	12 (24%)	16 (32%)
Pars intermedia, hyperplasia		1 (2%)		
Thyroid gland	(49)	(50)	(50)	(50)
C-cell, hyperplasia	40 (82%)	44 (88%)	45 (90%)	42 (84%)
Follicle, hyperplasia	2 (4%)	4 (8%)	7 (14%)	3 (6%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(48)	(49)	(50)
Cyst				2 (4%)
Fibrosis	1 (2%)			
Inflammation	11 (22%)	12 (25%)	13 (27%)	12 (24%)
Ovary	(50)	(50)	(50)	(50)
Cyst	9 (18%)	10 (20%)	12 (24%)	10 (20%)
Uterus	(50)	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)			
Thrombosis				1 (2%)
Cervix, hyperplasia		1 (2%)		
Endometrium, hyperplasia, cystic	12 (24%)	11 (22%)	12 (24%)	10 (20%)
Epithelium, metaplasia, squamous				1 (2%)
Vagina	(1)	(0)	(0)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(4)	(1)	(2)	(1)
Lumbar, hyperplasia, plasma cell	3 (75%)			
Popliteal, hyperplasia, plasma cell	1 (25%)			
Renal, hyperplasia, plasma cell	1 (25%)			

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Hematopoietic System (continued)				
Lymph node, bronchial	(37)	(37)	(44)	(36)
Hematopoietic cell proliferation	1 (3%)			
Hyperplasia, lymphohistiocytic	1 (3%)	18 (49%)	37 (84%)	31 (86%)
Hyperplasia, plasma cell	1 (3%)			
Lymph node, mandibular	(40)	(46)	(47)	(50)
Hematopoietic cell proliferation	1 (3%)			
Hyperplasia, plasma cell				1 (2%)
Lymph node, mediastinal	(46)	(49)	(46)	(45)
Hematopoietic cell proliferation	1 (2%)			
Hyperplasia, lymphohistiocytic		11 (22%)	14 (30%)	28 (62%)
Hyperplasia, plasma cell	5 (11%)		1 (2%)	1 (2%)
Pigmentation, hemosiderin				1 (2%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hyperplasia, plasma cell				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Cyst				1 (2%)
Hematopoietic cell proliferation	13 (26%)	7 (14%)	11 (22%)	9 (18%)
Lymphoid follicle, atrophy	2 (4%)	2 (4%)	6 (12%)	7 (14%)
Thymus	(48)	(49)	(49)	(49)
Atrophy	14 (29%)	12 (24%)	21 (43%)	15 (31%)
Cyst		1 (2%)	2 (4%)	
Ectopic thyroid	1 (2%)			
Hyperplasia, lymphohistiocytic			1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)		4 (8%)	3 (6%)
Duct, cyst	4 (8%)	7 (14%)	6 (12%)	2 (4%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)		1 (2%)	
Inflammation		1 (2%)	1 (2%)	1 (2%)
Ulcer	6 (12%)		3 (6%)	2 (4%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteopetrosis	1 (2%)			
Joint, inflammation	1 (2%)			
Skeletal muscle	(4)	(3)	(3)	(4)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	17 (34%)	16 (32%)	21 (42%)	17 (34%)
Degeneration	1 (2%)			
Hydrocephalus	6 (12%)	9 (18%)	4 (8%)	8 (16%)
Peripheral nerve	(4)	(3)	(2)	(3)
Spinal cord	(4)	(3)	(2)	(3)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Infiltration cellular, mixed cell		9 (18%)	18 (36%)	22 (44%)
Inflammation, chronic active		2 (4%)		1 (2%)
Epiglottis, hyperplasia, squamous	1 (2%)	24 (48%)	41 (82%)	50 (100%)
Epiglottis, metaplasia, squamous		49 (98%)	50 (100%)	50 (100%)
Lung	(50)	(50)	(50)	(50)
Fibrosis	5 (10%)	35 (70%)	49 (98%)	50 (100%)
Hyperplasia, lymphohistiocytic				1 (2%)
Infiltration cellular, histiocyte	16 (32%)	48 (96%)	50 (100%)	50 (100%)
Inflammation, chronic	1 (2%)			
Inflammation, chronic active	5 (10%)	46 (92%)	50 (100%)	50 (100%)
Alveolar/bronchiolar epithelium, hyperplasia	2 (4%)	9 (18%)	31 (62%)	50 (100%)
Alveolar epithelium, hyperplasia	8 (16%)	43 (86%)	49 (98%)	50 (100%)
Alveolar epithelium, metaplasia, squamous		3 (6%)	9 (18%)	21 (42%)
Alveolus, proteinosis	1 (2%)	15 (30%)	41 (82%)	48 (96%)
Bronchus-associated lymphoid tissue, hyperplasia, lymphohistiocytic		2 (4%)	7 (14%)	10 (20%)
Nose	(50)	(50)	(49)	(50)
Inflammation, suppurative	1 (2%)	46 (92%)	47 (96%)	48 (96%)
Glands, olfactory epithelium, hyperplasia		32 (64%)	43 (88%)	46 (92%)
Glands, olfactory epithelium, hyperplasia, squamous			1 (2%)	
Goblet cell, hyperplasia	3 (6%)	36 (72%)	40 (82%)	47 (94%)
Olfactory epithelium, accumulation, hyaline droplet	14 (28%)	50 (100%)	49 (100%)	50 (100%)
Olfactory epithelium, metaplasia, respiratory	1 (2%)			
Olfactory epithelium, metaplasia, squamous				2 (4%)
Respiratory epithelium, accumulation, hyaline droplet	5 (10%)	50 (100%)	48 (98%)	50 (100%)
Respiratory epithelium, hyperplasia		7 (14%)	8 (16%)	25 (50%)
Transitional epithelium, hyperplasia	5 (10%)	11 (22%)	4 (8%)	21 (42%)
Transitional epithelium, metaplasia, squamous	1 (2%)	5 (10%)	1 (2%)	2 (4%)
Trachea	(49)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract			1 (2%)	1 (2%)
Retina, atrophy	3 (6%)	2 (4%)	1 (2%)	2 (4%)
Harderian gland	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Inflammation	1 (2%)	3 (6%)	3 (6%)	2 (4%)
Zymbal's gland	(0)	(2)	(1)	(1)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hydronephrosis		2 (4%)		1 (2%)
Infarct	1 (2%)	2 (4%)		
Metaplasia, lipocyte		1 (2%)		
Nephropathy	4 (8%)	5 (10%)	5 (10%)	7 (14%)
Pelvis, inflammation	28 (56%)	32 (64%)	41 (82%)	30 (60%)
Renal tubule, cyst		1 (2%)	1 (2%)	3 (6%)
Renal tubule, hyperplasia		1 (2%)		
Ureter	(0)	(0)	(0)	(1)
Cyst				1 (100%)
Urinary bladder	(50)	(50)	(50)	(50)

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR INHALATION STUDY OF TRIM VX

TABLE C1 **Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of TRIM VX** **110**

TABLE C2 **Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of TRIM VX** **114**

TABLE C3 **Historical Incidence of Lung Neoplasms in Control Male B6C3F1/N Mice** **117**

TABLE C4 **Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of TRIM VX** **118**

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of TRIM VX[a]

	Chamber Control	10 mg/m³	30 mg/m³	100 mg/m³
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death			1	
Moribund	4	9	6	5
Natural deaths	8	2	6	8
Survivors				
Died last week of study			1	
Terminal kill	38	39	36	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(43)	(46)	(39)	(42)
Intestine large, cecum	(45)	(49)	(47)	(46)
Intestine large, colon	(48)	(50)	(48)	(48)
Intestine large, rectum	(46)	(48)	(46)	(45)
Intestine small, duodenum	(44)	(48)	(45)	(44)
Adenoma	2 (5%)			
Intestine small, ileum	(45)	(49)	(46)	(43)
Intestine small, jejunum	(45)	(48)	(45)	(44)
Liver	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)			
Fibrous histiocytoma				1 (2%)
Hemangiosarcoma, multiple			1 (2%)	
Hepatoblastoma			4 (8%)	2 (4%)
Hepatocellular adenoma	13 (26%)	11 (22%)	12 (24%)	12 (24%)
Hepatocellular adenoma, multiple	10 (20%)	18 (36%)	14 (28%)	24 (48%)
Hepatocellular carcinoma	10 (20%)	13 (26%)	11 (22%)	8 (16%)
Hepatocellular carcinoma, multiple	11 (22%)	5 (10%)	3 (6%)	13 (26%)
Hepatocholangiocarcinoma			1 (2%)	
Sarcoma, metastatic, mesentery			1 (2%)	
Mesentery	(5)	(7)	(4)	(2)
Hemangiosarcoma		1 (14%)	1 (25%)	
Hepatocellular carcinoma, metastatic, liver		2 (29%)		
Sarcoma			1 (25%)	
Pancreas	(50)	(50)	(49)	(50)
Fibrous histiocytoma				1 (2%)
Hepatocellular carcinoma, metastatic, liver		1 (2%)		
Salivary glands	(50)	(50)	(49)	(50)
Stomach, forestomach	(50)	(50)	(49)	(50)
Mast cell tumor malignant, metastatic, bone marrow	1 (2%)			
Squamous cell papilloma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Stomach, glandular	(48)	(50)	(49)	(50)
Sarcoma, metastatic, mesentery			1 (2%)	
Tooth	(5)	(10)	(8)	(5)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung			3 (6%)	
Endocardium, Schwannoma benign		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(49)
Adenoma	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Adenoma, multiple	1 (2%)			
Mast cell tumor malignant, metastatic, bone marrow	1 (2%)			
Subcapsular, adenoma		3 (6%)	1 (2%)	6 (12%)
Adrenal medulla	(50)	(50)	(48)	(49)
Islets, pancreatic	(50)	(50)	(48)	(50)
Parathyroid gland	(28)	(28)	(36)	(34)
Carcinoma				1 (3%)
Pituitary gland	(50)	(49)	(48)	(48)
Pars distalis, adenoma	1 (2%)			
Thyroid gland	(50)	(49)	(47)	(49)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(49)	(50)
Preputial gland	(49)	(50)	(48)	(49)
Prostate	(50)	(49)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(49)	(50)
Interstitial cell, adenoma		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(49)	(50)
Mast cell tumor malignant	1 (2%)			
Schwannoma malignant, metastatic, peripheral nerve			1 (2%)	
Lymph node	(3)	(0)	(3)	(1)
Pancreatic, sarcoma, metastatic, mesentery			1 (33%)	
Renal, fibrous histiocytoma				1 (100%)
Renal, sarcoma, metastatic, mesentery			1 (33%)	
Lymph node, bronchial	(38)	(37)	(39)	(39)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (3%)		2 (5%)	
Hepatoblastoma, metastatic, liver				1 (3%)
Hepatocellular carcinoma, metastatic, liver	1 (3%)			1 (3%)
Lymph node, mandibular	(20)	(27)	(25)	(27)
Lymph node, mediastinal	(35)	(35)	(41)	(40)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (3%)		1 (2%)	
Hepatoblastoma, metastatic, liver				1 (3%)
Mast cell tumor malignant, metastatic, bone marrow	1 (3%)			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Hematopoietic System (continued)				
Lymph node, mesenteric	(48)	(49)	(47)	(48)
Fibrous histiocytoma				1 (2%)
Hemangioma			1 (2%)	1 (2%)
Hemangiosarcoma				2 (4%)
Hepatoblastoma, metastatic, liver				1 (2%)
Sarcoma, metastatic, mesentery			1 (2%)	
Spleen	(49)	(50)	(49)	(50)
Thymus	(44)	(44)	(47)	(46)
Alveolar/bronchiolar carcinoma, metastatic, lung			2 (4%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Mast cell tumor malignant, metastatic, bone marrow	1 (2%)			
Integumentary System				
Mammary gland	(3)	(3)	(0)	(0)
Skin	(50)	(50)	(50)	(50)
Fibrosarcoma	1 (2%)			
Fibrous histiocytoma				1 (2%)
Subcutaneous tissue, hemangiosarcoma			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteoma		1 (2%)		
Skeletal muscle	(0)	(1)	(2)	(1)
Sarcoma, metastatic, mesentery			1 (50%)	
Schwannoma malignant, metastatic, peripheral nerve			1 (50%)	
Nervous System				
Brain	(50)	(50)	(49)	(50)
Peripheral nerve	(0)	(1)	(1)	(2)
Schwannoma malignant			1 (100%)	
Spinal cord	(0)	(1)	(1)	(2)
Respiratory System				
Larynx	(48)	(49)	(49)	(49)
Lung	(50)	(50)	(49)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	7 (14%)	5 (10%)	6 (12%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)	1 (2%)		3 (6%)
Alveolar/bronchiolar carcinoma	8 (16%)	8 (16%)	7 (14%)	9 (18%)
Alveolar/bronchiolar carcinoma, multiple	2 (4%)		2 (4%)	8 (16%)
Carcinoma, metastatic, Harderian gland				1 (2%)
Hepatoblastoma, metastatic, liver			1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	8 (16%)	5 (10%)	4 (8%)	6 (12%)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Schwannoma malignant, metastatic, peripheral nerve			1 (2%)	
Bronchiole, epithelium, adenoma			1 (2%)	
Mediastinum, hepatocellular carcinoma, metastatic, liver		1 (2%)		

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m³	30 mg/m³	100 mg/m³
Respiratory System (continued)				
Nose	(49)	(50)	(49)	(50)
Carcinoma, metastatic, Harderian gland				1 (2%)
Pleura	(0)	(1)	(0)	(1)
Trachea	(46)	(50)	(47)	(48)
Special Senses System				
Eye	(50)	(50)	(49)	(49)
Carcinoma, metastatic, Harderian gland				1 (2%)
Harderian gland	(50)	(50)	(49)	(50)
Adenoma	4 (8%)	5 (10%)	3 (6%)	6 (12%)
Adenoma, multiple				1 (2%)
Carcinoma	2 (4%)	3 (6%)	2 (4%)	1 (2%)
Urinary System				
Kidney	(50)	(50)	(49)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)
Mast cell tumor malignant, metastatic, bone marrow	1 (2%)			
Urethra	(0)	(0)	(1)	(0)
Urinary bladder	(50)	(50)	(49)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	1 (2%)	1 (2%)
Lymphoma malignant	6 (12%)	2 (4%)	3 (6%)	2 (4%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	42	42	42	48
Total primary neoplasms	81	83	78	113
Total animals with benign neoplasms	32	34	31	41
Total benign neoplasms	40	50	39	61
Total animals with malignant neoplasms	32	32	30	36
Total malignant neoplasms	41	33	39	52
Total animals with metastatic neoplasms	10	7	11	9
Total metastatic neoplasms	18	9	25	16

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Adrenal Cortex: Adenoma				
Overall rate[a]	3/50 (6%)	4/50 (8%)	2/49 (4%)	7/49 (14%)
Adjusted rate[b]	6.8%	8.8%	4.6%	15.7%
Terminal rate[c]	3/38 (8%)	4/39 (10%)	2/37 (5%)	7/37 (19%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test[d]	P=0.084	P=0.511	P=0.506N	P=0.158
Harderian Gland: Adenoma				
Overall rate	4/50 (8%)	5/50 (10%)	3/50 (6%)	7/50 (14%)
Adjusted rate	8.8%	11.0%	6.7%	15.4%
Terminal rate	3/38 (8%)	4/39 (10%)	2/37 (5%)	7/37 (19%)
First incidence (days)	414	656	647	729 (T)
Poly-3 test	P=0.195	P=0.505	P=0.503N	P=0.263
Harderian Gland: Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	4.5%	6.6%	4.5%	2.2%
Terminal rate	2/38 (5%)	3/39 (8%)	2/37 (5%)	1/37 (3%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.298N	P=0.509	P=0.692N	P=0.492N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	6/50 (12%)	8/50 (16%)	5/50 (10%)	8/50 (16%)
Adjusted rate	13.3%	17.5%	11.1%	17.6%
Terminal rate	5/38 (13%)	7/39 (18%)	4/37 (11%)	8/37 (22%)
First incidence (days)	414	656	647	729 (T)
Poly-3 test	P=0.398	P=0.392	P=0.504N	P=0.389
Liver: Hepatocellular Adenoma				
Overall rate	23/50 (46%)	29/50 (58%)	26/50 (52%)	36/50 (72%)
Adjusted rate	50.7%	62.7%	56.2%	74.8%
Terminal rate	20/38 (53%)	26/39 (67%)	22/37 (60%)	26/37 (70%)
First incidence (days)	618	628	344	537
Poly-3 test	P=0.014	P=0.166	P=0.373	P=0.011
Liver: Hepatocellular Carcinoma				
Overall rate	21/50 (42%)	18/50 (36%)	14/50 (28%)	21/50 (42%)
Adjusted rate	43.3%	38.2%	30.5%	42.9%
Terminal rate	12/38 (32%)	13/39 (33%)	9/37 (24%)	11/37 (30%)
First incidence (days)	414	550	583	424
Poly-3 test	P=0.436	P=0.382N	P=0.139N	P=0.563N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	34/50 (68%)	35/50 (70%)	33/50 (66%)	41/50 (82%)
Adjusted rate	69.6%	73.8%	70.3%	82.0%
Terminal rate	24/38 (63%)	29/39 (74%)	26/37 (70%)	28/37 (76%)
First incidence (days)	414	550	344	424
Poly-3 test	P=0.092	P=0.407	P=0.561	P=0.113
Liver: Hepatoblastoma				
Overall rate	0/50 (0%)	0/50 (0%)	4/50 (8%)	2/50 (4%)
Adjusted rate	0.0%	0.0%	8.8%	4.4%
Terminal rate	0/38 (0%)	0/39 (0%)	3/37 (8%)	1/37 (3%)
First incidence (days)	—[e]	—	549	571
Poly-3 test	P=0.216	—[f]	P=0.063	P=0.245

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	21/50 (42%)	18/50 (36%)	18/50 (36%)	23/50 (46%)
Adjusted rate	43.3%	38.2%	38.7%	46.5%
Terminal rate	12/38 (32%)	13/39 (33%)	12/37 (32%)	12/37 (32%)
First incidence (days)	414	550	549	424
Poly-3 test	P=0.302	P=0.382N	P=0.402N	P=0.457
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	34/50 (68%)	35/50 (70%)	35/50 (70%)	41/50 (82%)
Adjusted rate	69.6%	73.8%	73.6%	82.0%
Terminal rate	24/38 (63%)	29/39 (74%)	27/37 (73%)	28/37 (76%)
First incidence (days)	414	550	344	424
Poly-3 test	P=0.097	P=0.407	P=0.415	P=0.113
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	6/50 (12%)	8/50 (16%)	5/49 (10%)	9/50 (18%)
Adjusted rate	13.1%	17.5%	11.3%	19.6%
Terminal rate	2/38 (5%)	7/39 (18%)	4/37 (11%)	8/37 (22%)
First incidence (days)	549	656	533	571
Poly-3 test	P=0.279	P=0.382	P=0.522N	P=0.287
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	10/50 (20%)	8/50 (16%)	9/49 (18%)	17/50 (34%)
Adjusted rate	22.1%	17.7%	20.2%	36.5%
Terminal rate	7/38 (18%)	8/39 (21%)	7/37 (19%)	12/37 (32%)
First incidence (days)	549	729 (T)	533	571
Poly-3 test	P=0.021	P=0.394N	P=0.516N	P=0.097
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	14/50 (28%)	14/50 (28%)	11/49 (22%)	23/50 (46%)
Adjusted rate	30.5%	30.7%	24.7%	49.4%
Terminal rate	9/38 (24%)	13/39 (33%)	9/37 (24%)	18/37 (49%)
First incidence (days)	549	656	533	571
Poly-3 test	P=0.013	P=0.581	P=0.352N	P=0.047
All Organs: Hemangiosarcoma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	0.0%	2.2%	6.7%	4.4%
Terminal rate	0/38 (0%)	0/39 (0%)	3/37 (8%)	2/37 (5%)
First incidence (days)	—	644	729 (T)	729 (T)
Poly-3 test	P=0.282	P=0.505	P=0.120	P=0.242
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	0/50 (0%)	1/50 (2%)	4/50 (8%)	3/50 (6%)
Adjusted rate	0.0%	2.2%	9.0%	6.6%
Terminal rate	0/38 (0%)	0/39 (0%)	4/37 (11%)	3/37 (8%)
First incidence (days)	—	644	729 (T)	729 (T)
Poly-3 test	P=0.144	P=0.505	P=0.061	P=0.123
All Organs: Malignant Lymphoma				
Overall rate	6/50 (12%)	2/50 (4%)	3/50 (6%)	2/50 (4%)
Adjusted rate	13.4%	4.3%	6.6%	4.4%
Terminal rate	5/38 (13%)	1/39 (3%)	2/37 (5%)	2/37 (5%)
First incidence (days)	619	481	549	729 (T)
Poly-3 test	P=0.211N	P=0.124N	P=0.237N	P=0.128N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
All Organs: Benign Neoplasms				
Overall rate	32/50 (64%)	34/50 (68%)	31/50 (62%)	41/50 (82%)
Adjusted rate	68.0%	72.5%	65.7%	85.2%
Terminal rate	26/38 (68%)	29/39 (74%)	25/37 (68%)	31/37 (84%)
First incidence (days)	414	621	344	537
Poly-3 test	P=0.025	P=0.399	P=0.494N	P=0.034
All Organs: Malignant Neoplasms				
Overall rate	32/50 (64%)	32/50 (64%)	30/50 (60%)	36/50 (72%)
Adjusted rate	65.5%	66.1%	62.7%	72.5%
Terminal rate	22/38 (58%)	24/39 (62%)	21/37 (57%)	24/37 (65%)
First incidence (days)	414	481	533	424
Poly-3 test	P=0.233	P=0.560	P=0.470N	P=0.300
All Organs: Benign or Malignant Neoplasms				
Overall rate	42/50 (84%)	42/50 (84%)	42/50 (84%)	48/50 (96%)
Adjusted rate	86.0%	86.1%	85.9%	96.0%
Terminal rate	32/38 (84%)	33/39 (85%)	31/37 (84%)	35/37 (95%)
First incidence (days)	414	481	344	424
Poly-3 test	P=0.047	P=0.610	P=0.612N	P=0.077

(T) Terminal kill

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal cortex, liver, and lung; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE C3
Historical Incidence of Lung Neoplasms in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Alveolar/bronchiolar Adenoma	Alveolar/bronchiolar Carcinoma	Alveolar/bronchiolar Adenoma or Carcinoma	Bronchiole Epithelium Adenoma
Historical Incidence: Inhalation Studies				
Antimony trioxide (October 2008)	10/50	4/50	13/50	0/50
Cobalt metal (May 2006)	7/50	11/50	16/50	0/50
CIMSTAR 3800 (May 2008)	5/50	8/50	13/50	0/50
TRIM VX (August 2009)	6/50	10/50	14/50	0/50
Vinylidene chloride (June 2005)	7/50	9/50	13/50	0/50
Total (%)	35/250 (14.0%)	42/250 (16.8%)	69/250 (27.6%)	0/250
Mean ± standard deviation	14.0% ± 3.7%	16.8% ± 5.4%	27.6% ± 2.6%	
Range	10%-20%	8%-22%	26%-32%	
Overall Historical Incidence: All Routes				
Total (%)	83/550 (15.1%)	75/550 (13.6%)	147/550 (26.7%)	0/550
Mean ± standard deviation	15.1% ± 5.9%	13.6% ± 6.4%	26.7% ± 6.5%	
Range	8%-26%	4%-22%	16%-38%	

[a] Data as of July 2015

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of TRIM VX[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death			1	
Moribund	4	9	6	5
Natural deaths	8	2	6	8
Survivors				
Died last week of study			1	
Terminal kill	38	39	36	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(43)	(46)	(39)	(42)
Infiltration cellular, lymphoid			1 (3%)	
Inflammation, chronic active	1 (2%)	1 (2%)	1 (3%)	1 (2%)
Vacuolization cytoplasmic		1 (2%)		2 (5%)
Epithelium, hyperplasia				1 (2%)
Intestine large, cecum	(45)	(49)	(47)	(46)
Hemorrhage	1 (2%)			
Hyperplasia	1 (2%)			
Hyperplasia, lymphoid	2 (4%)			3 (7%)
Infiltration cellular, lymphoid			1 (2%)	
Inflammation	2 (4%)	2 (4%)	3 (6%)	1 (2%)
Necrosis	1 (2%)		1 (2%)	
Intestine large, colon	(48)	(50)	(48)	(48)
Hyperplasia, lymphoid			1 (2%)	
Intestine large, rectum	(46)	(48)	(46)	(45)
Inflammation		1 (2%)		
Intestine small, duodenum	(44)	(48)	(45)	(44)
Inflammation				1 (2%)
Intestine small, ileum	(45)	(49)	(46)	(43)
Cyst		1 (2%)		
Hyperplasia, lymphoid	8 (18%)	9 (18%)	11 (24%)	11 (26%)
Inflammation		1 (2%)	1 (2%)	
Intestine small, jejunum	(45)	(48)	(45)	(44)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	3 (7%)	2 (5%)
Inflammation			1 (2%)	
Necrosis			1 (2%)	
Perforation			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Basophilic focus	11 (22%)	9 (18%)	3 (6%)	4 (8%)
Clear cell focus	14 (28%)	11 (22%)	9 (18%)	13 (26%)
Congestion	1 (2%)			
Eosinophilic focus	8 (16%)	12 (24%)	17 (34%)	7 (14%)
Fatty change				1 (2%)
Fibrosis			1 (2%)	
Hemorrhage	1 (2%)			
Inflammation, chronic active			2 (4%)	1 (2%)
Mixed cell focus	3 (6%)	3 (6%)	6 (12%)	5 (10%)
Necrosis				1 (2%)
Tension lipidosis	1 (2%)	3 (6%)	3 (6%)	
Thrombosis				1 (2%)
Bile duct, vacuolization cytoplasmic				1 (2%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Alimentary System (continued)				
Liver (continued)	(50)	(50)	(50)	(50)
Centrilobular, hepatocyte, fatty change		2 (4%)		
Centrilobular, hepatocyte, necrosis	2 (4%)			
Hepatocyte, hypertrophy	1 (2%)	1 (2%)		1 (2%)
Hepatocyte, necrosis	3 (6%)	7 (14%)	5 (10%)	5 (10%)
Hepatocyte, vacuolization cytoplasmic	1 (2%)			
Periportal, fatty change			1 (2%)	
Serosa, fibrosis			1 (2%)	
Mesentery	(5)	(7)	(4)	(2)
Fat, necrosis	4 (80%)	4 (57%)	1 (25%)	2 (100%)
Pancreas	(50)	(50)	(49)	(50)
Atrophy			1 (2%)	
Cyst				1 (2%)
Hemorrhage, acute		1 (2%)		
Hypertrophy	2 (4%)			
Inflammation, chronic active		3 (6%)		
Acinus, atrophy				1 (2%)
Duct, hyperplasia		1 (2%)		
Salivary glands	(50)	(50)	(49)	(50)
Stomach, forestomach	(50)	(50)	(49)	(50)
Abscess				1 (2%)
Cyst, squamous				1 (2%)
Hyperplasia		1 (2%)	5 (10%)	1 (2%)
Infiltration cellular, lymphoid		1 (2%)		
Inflammation, chronic active	1 (2%)	3 (6%)	3 (6%)	5 (10%)
Necrosis		1 (2%)	1 (2%)	
Stomach, glandular	(48)	(50)	(49)	(50)
Cyst, squamous	1 (2%)			
Infiltration cellular, lymphoid			1 (2%)	
Inflammation, chronic active	1 (2%)	4 (8%)	2 (4%)	4 (8%)
Mineralization	1 (2%)	1 (2%)	2 (4%)	
Tooth	(5)	(10)	(8)	(5)
Dysplasia	4 (80%)	9 (90%)	7 (88%)	3 (60%)
Inflammation, suppurative, chronic active		2 (20%)		
Inflammation, suppurative			1 (13%)	2 (40%)
Malformation	1 (20%)			
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	19 (38%)	17 (34%)	18 (36%)	19 (38%)
Inflammation, chronic active	1 (2%)	3 (6%)		1 (2%)
Mineralization	1 (2%)			
Atrium, thrombosis	2 (4%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(49)
Accessory adrenal cortical nodule		1 (2%)	1 (2%)	1 (2%)
Angiectasis			1 (2%)	2 (4%)
Hyperplasia	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Hypertrophy	21 (42%)	30 (60%)	26 (53%)	18 (37%)
Subcapsular, hyperplasia		1 (2%)		
Subcapsular, hypertrophy		1 (2%)		

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Endocrine System (continued)				
Adrenal medulla	(50)	(50)	(48)	(49)
Angiectasis	1 (2%)	2 (4%)		1 (2%)
Hyperplasia	6 (12%)	5 (10%)	5 (10%)	5 (10%)
Hypertrophy	1 (2%)			1 (2%)
Islets, pancreatic	(50)	(50)	(48)	(50)
Hyperplasia		2 (4%)		
Hypertrophy			1 (2%)	
Parathyroid gland	(28)	(28)	(36)	(34)
Cyst		1 (4%)		1 (3%)
Pituitary gland	(50)	(49)	(48)	(48)
Pars distalis, angiectasis			1 (2%)	1 (2%)
Pars distalis, cyst	4 (8%)	1 (2%)	3 (6%)	6 (13%)
Pars distalis, hyperplasia	3 (6%)	3 (6%)	2 (4%)	3 (6%)
Pars intermedia, cyst	1 (2%)			
Thyroid gland	(50)	(49)	(47)	(49)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(49)	(50)
Exfoliated germ cell	39 (78%)	40 (80%)	44 (90%)	40 (80%)
Infiltration cellular, lymphoid				1 (2%)
Inflammation, chronic active	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Preputial gland	(49)	(50)	(48)	(49)
Ectasia	4 (8%)	3 (6%)	3 (6%)	4 (8%)
Inflammation, chronic active	9 (18%)	14 (28%)	12 (25%)	12 (24%)
Necrosis			1 (2%)	
Duct, ectasia		2 (4%)		
Prostate	(50)	(49)	(50)	(50)
Hyperplasia			3 (6%)	
Infiltration cellular, lymphoid	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Inflammation	2 (4%)	1 (2%)	1 (2%)	
Arteriole, inflammation, chronic active	1 (2%)			
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation				1 (2%)
Infiltration cellular, lymphoid	1 (2%)	1 (2%)	1 (2%)	
Inflammation	1 (2%)	2 (4%)	1 (2%)	
Testes	(50)	(50)	(49)	(50)
Degeneration	9 (18%)	17 (34%)	15 (31%)	9 (18%)
Necrosis		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(49)	(50)
Necrosis		2 (4%)		
Myeloid cell, hyperplasia	16 (32%)	29 (58%)	18 (37%)	29 (58%)
Lymph node	(3)	(0)	(3)	(1)
Lumbar, hyperplasia, lymphoid			1 (33%)	
Pancreatic, hyperplasia, lymphoid	1 (33%)			

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Hematopoietic System (continued)				
Lymph node, bronchial	(38)	(37)	(39)	(39)
Hematopoietic cell proliferation		1 (3%)		
Hyperplasia, lymphoid	3 (8%)	3 (8%)	2 (5%)	14 (36%)
Infiltration cellular, histiocyte		1 (3%)		7 (18%)
Infiltration cellular, plasma cell				1 (3%)
Plasma cell, hyperplasia			1 (3%)	
Lymph node, mandibular	(20)	(27)	(25)	(27)
Hematopoietic cell proliferation	1 (5%)			
Hyperplasia, lymphoid	3 (15%)	3 (11%)	2 (8%)	1 (4%)
Infiltration cellular, histiocyte	1 (5%)	1 (4%)	1 (4%)	1 (4%)
Lymph node, mediastinal	(35)	(35)	(41)	(40)
Hematopoietic cell proliferation			1 (2%)	
Hyperplasia, lymphoid	3 (9%)	2 (6%)	1 (2%)	5 (13%)
Lymph node, mesenteric	(48)	(49)	(47)	(48)
Angiectasis	1 (2%)		3 (6%)	
Congestion	1 (2%)			
Hematopoietic cell proliferation		3 (6%)	4 (9%)	1 (2%)
Hyperplasia, lymphoid	15 (31%)	11 (22%)	9 (19%)	6 (13%)
Hyperplasia, plasma cell	1 (2%)		1 (2%)	
Infiltration cellular		1 (2%)		
Inflammation, chronic active		1 (2%)	1 (2%)	1 (2%)
Spleen	(49)	(50)	(49)	(50)
Angiectasis		1 (2%)		
Hematopoietic cell proliferation	11 (22%)	8 (16%)	11 (22%)	10 (20%)
Hyperplasia, lymphoid	13 (27%)	15 (30%)	9 (18%)	8 (16%)
Necrosis, lymphoid		1 (2%)		
Thymus	(44)	(44)	(47)	(46)
Atrophy	5 (11%)	5 (11%)	8 (17%)	9 (20%)
Cyst	1 (2%)	3 (7%)	5 (11%)	5 (11%)
Ectopic parathyroid gland	1 (2%)	2 (5%)	3 (6%)	
Ectopic thyroid				1 (2%)
Hyperplasia, lymphoid	1 (2%)	5 (11%)		4 (9%)
Epithelial cell, hyperplasia	1 (2%)			
Integumentary System				
Mammary gland	(3)	(3)	(0)	(0)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		4 (8%)
Fibrosis			1 (2%)	
Hyperkeratosis				1 (2%)
Hyperplasia	3 (6%)	2 (4%)		3 (6%)
Inflammation, chronic active	10 (20%)	6 (12%)	8 (16%)	11 (22%)
Metaplasia, osseous			1 (2%)	
Necrosis	1 (2%)	3 (6%)	1 (2%)	
Ulcer	3 (6%)	2 (4%)	3 (6%)	3 (6%)
Hair follicle, atrophy			1 (2%)	
Subcutaneous tissue, inflammation, chronic active		1 (2%)		

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m³	30 mg/m³	100 mg/m³
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	1 (2%)	3 (6%)	1 (2%)	
Hyperostosis	1 (2%)	1 (2%)		
Skeletal muscle	(0)	(1)	(2)	(1)
Degeneration		1 (100%)		1 (100%)
Nervous System				
Brain	(50)	(50)	(49)	(50)
Compression			1 (2%)	
Gliosis				1 (2%)
Infiltration cellular	1 (2%)	2 (4%)	1 (2%)	
Inflammation, chronic active	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Peripheral nerve	(0)	(1)	(1)	(2)
Degeneration		1 (100%)		1 (50%)
Spinal cord	(0)	(1)	(1)	(2)
Respiratory System				
Larynx	(48)	(49)	(49)	(49)
Foreign body		1 (2%)		
Inflammation, chronic active	45 (94%)	47 (96%)	46 (94%)	48 (98%)
Mineralization			1 (2%)	
Necrosis		1 (2%)	1 (2%)	
Epiglottis, hyperplasia, squamous	1 (2%)	2 (4%)	14 (29%)	30 (61%)
Epiglottis, metaplasia, squamous		49 (100%)	49 (100%)	49 (100%)
Squamous epithelium, hyperplasia	1 (2%)			
Lung	(50)	(50)	(49)	(50)
Fibrosis		2 (4%)	5 (10%)	45 (90%)
Hemorrhage		1 (2%)	1 (2%)	
Infiltration cellular, histiocyte	5 (10%)	9 (18%)	15 (31%)	49 (98%)
Inflammation, chronic	5 (10%)	12 (24%)	16 (33%)	50 (100%)
Metaplasia, osseous				1 (2%)
Pigmentation	2 (4%)	2 (4%)	2 (4%)	8 (16%)
Proteinosis			1 (2%)	
Thrombosis		1 (2%)	2 (4%)	
Alveolar/bronchiolar epithelium, hyperplasia	3 (6%)	7 (14%)	15 (31%)	50 (100%)
Alveolar epithelium, hyperplasia	3 (6%)	3 (6%)	7 (14%)	47 (94%)
Bronchiole, metaplasia, squamous				1 (2%)
Nose	(49)	(50)	(49)	(50)
Exudate	2 (4%)	11 (22%)	35 (71%)	49 (98%)
Inflammation, suppurative	1 (2%)			
Inflammation, chronic			1 (2%)	
Inflammation, chronic active	3 (6%)	33 (66%)	39 (80%)	50 (100%)
Polyp, inflammatory			1 (2%)	
Glands, olfactory epithelium, hyperplasia				1 (2%)
Lateral wall, inflammation, chronic active	2 (4%)	7 (14%)	4 (8%)	5 (10%)
Lateral wall, necrosis		1 (2%)		
Nasolacrimal duct, abscess	1 (2%)			
Nasolacrimal duct, inflammation, chronic active	3 (6%)	2 (4%)	2 (4%)	2 (4%)
Nasopharyngeal duct, hyperplasia		1 (2%)		
Nasopharyngeal duct, inflammation, chronic active		1 (2%)		

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Respiratory System (continued)				
Nose (continued)	(49)	(50)	(49)	(50)
Nasopharyngeal duct, perforation		1 (2%)	11 (22%)	19 (38%)
Olfactory epithelium, accumulation, hyaline droplet	2 (4%)	46 (92%)	48 (98%)	50 (100%)
Olfactory epithelium, atrophy		1 (2%)		4 (8%)
Olfactory epithelium, metaplasia, respiratory	3 (6%)	2 (4%)	3 (6%)	5 (10%)
Respiratory epithelium, accumulation, hyaline droplet	7 (14%)	49 (98%)	49 (100%)	50 (100%)
Respiratory epithelium, atrophy		1 (2%)	20 (41%)	40 (80%)
Respiratory epithelium, hyperplasia	41 (84%)	49 (98%)	47 (96%)	48 (96%)
Respiratory epithelium, metaplasia, squamous	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Respiratory epithelium, necrosis	2 (4%)	1 (2%)	2 (4%)	23 (46%)
Turbinate, atrophy		2 (4%)	5 (10%)	14 (28%)
Turbinate, perforation			1 (2%)	13 (26%)
Pleura	(0)	(1)	(0)	(1)
Trachea	(46)	(50)	(47)	(48)
Cartilage, metaplasia, osseous	1 (2%)		1 (2%)	1 (2%)
Epithelium, hyperplasia				3 (6%)
Special Senses System				
Eye	(50)	(50)	(49)	(49)
Cataract				1 (2%)
Inflammation, chronic active	1 (2%)	4 (8%)	3 (6%)	3 (6%)
Necrosis			1 (2%)	
Harderian gland	(50)	(50)	(49)	(50)
Hyperplasia	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Inflammation		1 (2%)		
Urinary System				
Kidney	(50)	(50)	(49)	(50)
Angiectasis			1 (2%)	
Cyst			1 (2%)	1 (2%)
Hydronephrosis				1 (2%)
Hyperplasia, lymphoid	1 (2%)	2 (4%)		
Infarct, chronic	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Inflammation, chronic active	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Metaplasia, osseous		2 (4%)	1 (2%)	1 (2%)
Mineralization	1 (2%)	1 (2%)		
Nephropathy	47 (94%)	46 (92%)	45 (92%)	49 (98%)
Artery, inflammation, chronic active			1 (2%)	
Urethra	(0)	(0)	(1)	(0)
Urinary bladder	(50)	(50)	(49)	(50)
Angiectasis		1 (2%)		
Calculus microscopic observation only	1 (2%)			
Inflammation, suppurative		1 (2%)		
Inflammation, chronic active			1 (2%)	
Ulcer		1 (2%)		

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF TRIM VX

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TABLE D2 Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of TRIM VX 130

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TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of TRIM VX[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	1			
Moribund	10	9	4	13
Natural deaths	4	5	10	7
Survivors				
Died last week of study	1			1
Terminal kill	34	36	36	29
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(49)
Gallbladder	(46)	(46)	(44)	(44)
Intestine large, cecum	(50)	(49)	(46)	(45)
Intestine large, colon	(50)	(50)	(47)	(50)
Intestine large, rectum	(48)	(48)	(46)	(45)
Intestine small, duodenum	(48)	(46)	(43)	(45)
Intestine small, ileum	(48)	(47)	(44)	(46)
Intestine small, jejunum	(48)	(45)	(45)	(46)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, islets, pancreatic				1 (2%)
Hepatoblastoma	1 (2%)			
Hepatocellular adenoma	7 (14%)	11 (22%)	7 (14%)	9 (18%)
Hepatocellular adenoma, multiple	2 (4%)	4 (8%)	3 (6%)	6 (12%)
Hepatocellular carcinoma	7 (14%)	2 (4%)	5 (10%)	9 (18%)
Hepatocellular carcinoma, multiple		3 (6%)	2 (4%)	3 (6%)
Ito cell tumor benign		1 (2%)		
Mesentery	(10)	(10)	(5)	(11)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (9%)
Hemangioma				1 (9%)
Schwannoma malignant, metastatic, heart				1 (9%)
Pancreas	(50)	(50)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Sarcoma, metastatic, skin				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Mast cell tumor benign			1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)			
Stomach, glandular	(49)	(50)	(49)	(50)
Tooth	(0)	(1)	(0)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Aorta, hemangiosarcoma	1 (2%)			
Heart	(50)	(50)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				2 (4%)
Endocardium, schwannoma malignant				1 (2%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Subcapsular, adenoma				1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	2 (4%)	1 (2%)	1 (2%)	
Pheochromocytoma malignant		1 (2%)		
Islets, pancreatic	(50)	(49)	(49)	(50)
Adenoma			1 (2%)	
Carcinoma				1 (2%)
Parathyroid gland	(35)	(30)	(36)	(28)
Adenoma	1 (3%)			
Pituitary gland	(48)	(49)	(49)	(49)
Pars distalis, adenoma	8 (17%)	6 (12%)	7 (14%)	6 (12%)
Pars distalis, carcinoma	3 (6%)			2 (4%)
Thyroid gland	(50)	(50)	(50)	(49)
C-cell, carcinoma	1 (2%)			
Follicular cell, adenoma			1 (2%)	
Follicular cell, carcinoma, multiple				1 (2%)
General Body System				
Tissue NOS	(1)	(0)	(0)	(0)
Genital System				
Clitoral gland	(46)	(48)	(45)	(46)
Ovary	(50)	(49)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Cystadenoma	1 (2%)	4 (8%)	2 (4%)	3 (6%)
Granulosa cell tumor benign	1 (2%)			1 (2%)
Periovarian tissue, alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Periovarian tissue, sarcoma, metastatic, skin				1 (2%)
Uterus	(50)	(50)	(50)	(50)
Carcinoma				1 (2%)
Hemangioma		1 (2%)		1 (2%)
Hemangiosarcoma	1 (2%)	2 (4%)		
Polyp stromal	1 (2%)	3 (6%)	5 (10%)	1 (2%)
Endometrium, adenoma			1 (2%)	
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Hemangiosarcoma, metastatic, spleen			1 (2%)	
Lymph node	(11)	(11)	(11)	(10)
Axillary, alveolar/bronchiolar carcinoma, metastatic, lung				1 (10%)
Axillary, schwannoma malignant, metastatic, skin		1 (9%)		
Lumbar, hemangiosarcoma	1 (9%)			
Pancreatic, hemangiosarcoma				1 (10%)
Renal, alveolar/bronchiolar carcinoma, metastatic, lung				1 (10%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m³	30 mg/m³	100 mg/m³
Hematopoietic System (continued)				
Lymph node, bronchial	(44)	(44)	(44)	(43)
Alveolar/bronchiolar carcinoma, metastatic, lung				4 (9%)
Carcinoma, metastatic, Harderian gland				1 (2%)
Carcinoma, metastatic, mammary gland		1 (2%)		
Lymph node, mandibular	(35)	(42)	(37)	(39)
Lymph node, mediastinal	(40)	(44)	(45)	(45)
Alveolar/bronchiolar carcinoma, metastatic, lung				3 (7%)
Schwannoma malignant, metastatic, skin		1 (2%)		
Lymph node, mesenteric	(49)	(49)	(47)	(48)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Hemangioma	1 (2%)			
Sarcoma, metastatic, skin				1 (2%)
Spleen	(50)	(49)	(48)	(49)
Hemangiosarcoma		1 (2%)	2 (4%)	
Thymus	(48)	(48)	(47)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung				2 (4%)
Sarcoma, metastatic, skin				1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	1 (2%)	
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Carcinoma		1 (2%)	1 (2%)	
Skin	(50)	(50)	(50)	(50)
Schwannoma benign				1 (2%)
Schwannoma malignant			1 (2%)	
Subcutaneous tissue, fibrous histiocytoma		1 (2%)		1 (2%)
Subcutaneous tissue, hemangiosarcoma			1 (2%)	
Subcutaneous tissue, sarcoma				1 (2%)
Subcutaneous tissue, schwannoma malignant		3 (6%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hemangiosarcoma, metastatic, spleen			1 (2%)	
Mandible, fibrosarcoma				1 (2%)
Skeletal muscle	(3)	(4)	(2)	(4)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (25%)
Sarcoma				1 (25%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, mammary gland		1 (2%)		
Peripheral nerve	(3)	(3)	(1)	(2)
Spinal cord	(2)	(3)	(1)	(2)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	4 (8%)	5 (10%)	3 (6%)	6 (12%)
Alveolar/bronchiolar adenoma, multiple				2 (4%)
Alveolar/bronchiolar carcinoma	3 (6%)	3 (6%)	5 (10%)	9 (18%)
Alveolar/bronchiolar carcinoma, multiple	2 (4%)		1 (2%)	5 (10%)
Carcinoma, metastatic, Harderian gland				1 (2%)
Carcinoma, metastatic, mammary gland		1 (2%)		
Carcinoma, metastatic, thyroid gland	1 (2%)			
Hepatocellular carcinoma, metastatic, liver		2 (4%)	2 (4%)	3 (6%)
Pheochromocytoma malignant, metastatic, adrenal medulla		1 (2%)		
Sarcoma, metastatic, skin				1 (2%)
Nose	(50)	(50)	(50)	(50)
Carcinoma, metastatic, Harderian gland				1 (2%)
Trachea	(49)	(50)	(50)	(49)
Special Senses System				
Ear	(0)	(0)	(0)	(1)
Eye	(49)	(50)	(50)	(50)
Harderian gland	(50)	(49)	(49)	(49)
Adenoma	1 (2%)	1 (2%)	5 (10%)	4 (8%)
Carcinoma	3 (6%)	2 (4%)	2 (4%)	5 (10%)
Urinary System				
Kidney	(49)	(50)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				2 (4%)
Carcinoma, metastatic, mammary gland		1 (2%)		
Pheochromocytoma malignant, metastatic, adrenal medulla		1 (2%)		
Sarcoma, metastatic, skin				1 (2%)
Schwannoma malignant, metastatic, heart				1 (2%)
Urethra	(0)	(1)	(0)	(0)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	4 (8%)	5 (10%)	3 (6%)	3 (6%)
Lymphoma malignant	10 (20%)	8 (16%)	14 (28%)	12 (24%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	38	41	42	46
Total primary neoplasms	67	70	75	99
Total animals with benign neoplasms	23	22	28	30
Total benign neoplasms	30	38	38	42
Total animals with malignant neoplasms	27	29	32	37
Total malignant neoplasms	37	32	37	57
Total animals with metastatic neoplasms	7	8	5	11
Total metastatic neoplasms	9	15	7	42

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Harderian Gland: Adenoma				
Overall rate[a]	1/50 (2%)	1/50 (2%)	5/50 (10%)	4/50 (8%)
Adjusted rate[b]	2.3%	2.2%	10.9%	9.2%
Terminal rate[c]	0/35 (0%)	1/36 (3%)	4/36 (11%)	2/30 (7%)
First incidence (days)	683	731 (T)	716	645
Poly-3 test[d]	P=0.122	P=0.758N	P=0.110	P=0.175
Harderian Gland: Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	2/50 (4%)	5/50 (10%)
Adjusted rate	6.8%	4.5%	4.4%	11.5%
Terminal rate	3/35 (9%)	2/36 (6%)	2/36 (6%)	3/30 (10%)
First incidence (days)	731 (T)	731 (T)	731 (T)	661
Poly-3 test	P=0.163	P=0.492N	P=0.481N	P=0.352
Harderian Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	7/50 (14%)	9/50 (18%)
Adjusted rate	9.1%	6.7%	15.3%	20.5%
Terminal rate	3/35 (9%)	3/36 (8%)	6/36 (17%)	5/30 (17%)
First incidence (days)	683	731 (T)	716	645
Poly-3 test	P=0.039	P=0.492N	P=0.283	P=0.112
Liver: Hepatocellular Adenoma				
Overall rate	9/50 (18%)	15/50 (30%)	10/50 (20%)	15/50 (30%)
Adjusted rate	20.5%	33.5%	21.6%	33.8%
Terminal rate	8/35 (23%)	14/36 (39%)	8/36 (22%)	10/30 (33%)
First incidence (days)	721	721	649	572
Poly-3 test	P=0.197	P=0.124	P=0.551	P=0.120
Liver: Hepatocellular Carcinoma				
Overall rate	7/50 (14%)[e]	5/50 (10%)	7/50 (14%)	12/50 (24%)
Adjusted rate	15.8%	11.1%	15.2%	26.7%
Terminal rate	5/35 (14%)	3/36 (8%)	4/36 (11%)	4/30 (13%)
First incidence (days)	645	607	649	557
Poly-3 test	P=0.042	P=0.364N	P=0.581N	P=0.158
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	15/50 (30%)[e]	19/50 (38%)	15/50 (30%)	24/50 (48%)
Adjusted rate	33.9%	42.1%	32.3%	52.3%
Terminal rate	13/35 (37%)	16/36 (44%)	11/36 (31%)	13/30 (43%)
First incidence (days)	645	607	649	557
Poly-3 test	P=0.049	P=0.281	P=0.525N	P=0.056
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	5/50 (10%)	3/50 (6%)	8/50 (16%)
Adjusted rate	9.0%	11.2%	6.6%	18.3%
Terminal rate	2/35 (6%)	5/36 (14%)	3/36 (8%)	5/30 (17%)
First incidence (days)	575	731 (T)	731 (T)	668
Poly-3 test	P=0.100	P=0.502	P=0.486N	P=0.166
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	5/50 (10%)	3/50 (6%)	6/50 (12%)	14/50 (28%)
Adjusted rate	11.4%	6.7%	13.1%	31.4%
Terminal rate	5/35 (14%)	2/36 (6%)	6/36 (17%)	8/30 (27%)
First incidence (days)	731 (T)	647	731 (T)	513
Poly-3 test	P<0.001	P=0.342N	P=0.529	P=0.018

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	9/50 (18%)	8/50 (16%)	8/50 (16%)	20/50 (40%)
Adjusted rate	20.2%	17.8%	17.5%	44.5%
Terminal rate	7/35 (20%)	7/36 (19%)	8/36 (22%)	12/30 (40%)
First incidence (days)	575	647	731 (T)	513
Poly-3 test	P<0.001	P=0.491N	P=0.476N	P=0.011
Ovary: Cystadenoma				
Overall rate	1/50 (2%)	4/49 (8%)	2/49 (4%)	3/50 (6%)
Adjusted rate	2.3%	9.2%	4.5%	6.9%
Terminal rate	1/35 (3%)	4/35 (11%)	1/36 (3%)	3/30 (10%)
First incidence (days)	731 (T)	731 (T)	725	731 (T)
Poly-3 test	P=0.446	P=0.177	P=0.507	P=0.300
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	8/48 (17%)	6/49 (12%)	7/49 (14%)	6/49 (12%)
Adjusted rate	18.7%	13.4%	15.6%	14.0%
Terminal rate	6/35 (17%)	5/36 (14%)	6/36 (17%)	5/29 (17%)
First incidence (days)	647	647	722	557
Poly-3 test	P=0.437N	P=0.351N	P=0.463N	P=0.387N
Pituitary Gland (Pars Distalis): Carcinoma				
Overall rate	3/48 (6%)	0/49 (0%)	0/49 (0%)	2/49 (4%)
Adjusted rate	7.0%	0.0%	0.0%	4.7%
Terminal rate	2/35 (6%)	0/36 (0%)	0/36 (0%)	2/29 (7%)
First incidence (days)	522	—[f]	—	731 (T)
Poly-3 test	P=0.536	P=0.113N	P=0.112N	P=0.510N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	11/48 (23%)	6/49 (12%)	7/49 (14%)	8/49 (16%)
Adjusted rate	25.3%	13.4%	15.6%	18.7%
Terminal rate	8/35 (23%)	5/36 (14%)	6/36 (17%)	7/29 (24%)
First incidence (days)	522	647	722	557
Poly-3 test	P=0.499N	P=0.124N	P=0.194N	P=0.316N
Skin: Malignant Schwannoma				
Overall rate	0/50 (%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	6.5%	2.2%	0.0%
Terminal rate	0/35 (0%)	1/36 (3%)	0/36 (0%)	0/30 (0%)
First incidence (days)	—	479	655	—
Poly-3 test	P=0.278N	P=0.127	P=0.509	—[g]
Skin: Benign or Malignant Schwannoma				
Overall rate	0/50 (%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	0.0%	6.5%	2.2%	2.3%
Terminal rate	0/35 (0%)	1/36 (3%)	0/36 (0%)	1/30 (3%)
First incidence (days)	—	479	655	731 (T)
Poly-3 test	P=0.592N	P=0.127	P=0.509	P=0.497
Uterus: Stromal Polyp				
Overall rate	1/50 (2%)	3/50 (6%)	5/50 (10%)	1/50 (2%)
Adjusted rate	2.3%	6.7%	10.9%	2.3%
Terminal rate	1/35 (3%)	2/36 (6%)	4/36 (11%)	1/30 (3%)
First incidence (days)	731 (T)	721	715	731 (T)
Poly-3 test	P=0.401N	P=0.312	P=0.111	P=0.758

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
All Organs: Hemangiosarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.5%	6.7%	6.5%	2.3%
Terminal rate	1/35 (3%)	2/36 (6%)	1/36 (3%)	1/30 (3%)
First incidence (days)	647	640	637	731 (T)
Poly-3 test	P=0.314N	P=0.508	P=0.519	P=0.508N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	3/50 (6%)	4/50 (8%)	3/50 (6%)	3/50 (6%)
Adjusted rate	6.8%	8.9%	6.5%	6.9%
Terminal rate	1/35 (3%)	3/36 (8%)	1/36 (3%)	2/30 (7%)
First incidence (days)	647	640	637	673
Poly-3 test	P=0.544N	P=0.507	P=0.645N	P=0.653
All Organs: Histiocytic Sarcoma				
Overall rate	4/50 (8%)	5/50 (10%)	3/50 (6%)	3/50 (6%)
Adjusted rate	8.9%	11.1%	6.6%	6.9%
Terminal rate	1/35 (3%)	3/36 (8%)	3/36 (8%)	0/30 (0%)
First incidence (days)	550	688	731 (T)	645
Poly-3 test	P=0.381N	P=0.499	P=0.492N	P=0.516N
All Organs: Malignant Lymphoma				
Overall rate	10/50 (20%)	8/50 (16%)	14/50 (28%)	12/50 (24%)
Adjusted rate	22.2%	17.6%	30.1%	27.1%
Terminal rate	7/35 (20%)	7/36 (19%)	10/36 (28%)	8/30 (27%)
First incidence (days)	575	365	619	572
Poly-3 test	P=0.261	P=0.386N	P=0.266	P=0.387
All Organs: Benign Neoplasms				
Overall rate	23/50 (46%)	22/50 (44%)	28/50 (56%)	30/50 (60%)
Adjusted rate	50.8%	48.5%	60.2%	65.5%
Terminal rate	17/35 (49%)	19/36 (53%)	22/36 (61%)	20/30 (67%)
First incidence (days)	575	640	649	557
Poly-3 test	P=0.056	P=0.497N	P=0.238	P=0.107
All Organs: Malignant Neoplasms				
Overall rate	27/50 (54%)	29/50 (58%)	32/50 (64%)	37/50 (74%)
Adjusted rate	57.2%	59.6%	67.3%	76.8%
Terminal rate	18/35 (51%)	19/36 (53%)	22/36 (61%)	21/30 (70%)
First incidence (days)	522	365	619	319
Poly-3 test	P=0.019	P=0.488	P=0.209	P=0.030

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
All Organs: Benign or Malignant Neoplasms				
Overall rate	38/50 (76%)	41/50 (82%)	42/50 (84%)	46/50 (92%)
Adjusted rate	80.0%	84.2%	88.2%	94.7%
Terminal rate	27/35 (77%)	30/36 (83%)	31/36 (86%)	28/30 (93%)
First incidence (days)	522	365	619	319
Poly-3 test	P=0.022	P=0.389	P=0.198	P=0.024

(T) Terminal kill
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, and pituitary gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] A single incidence of hepatoblastoma occurred in an animal that also had a hepatocellular carcinoma.
[f] Not applicable; no neoplasms in animal group
[g] Value of statistic cannot be computed.

TABLE D3
Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Female B6C3F1/N Mice[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Inhalation Studies			
Antimony trioxide (October 2008)	1/50	2/50	3/50
Cobalt metal (May 2006)	3/49	5/49	8/49
CIMSTAR 3800 (May 2008)	1/50	4/50	4/50
TRIM VX (August 2009)	4/50	5/50	9/50
Vinylidene chloride (June 2005)	3/50	1/50	4/50
Total (%)	12/249 (4.8%)	17/249 (6.8%)	28/249 (11.2%)
Mean ± standard deviation	4.8% ± 2.7%	6.8% ± 3.7%	11.3% ± 5.5%
Range	2%-8%	2%-10%	6%-18%
Overall Historical Incidence: All Routes			
Total (%)	27/549 (4.9%)	24/549 (4.4%)	50/549 (9.1%)
Mean ± standard deviation	4.9% ± 3.5%	4.4% ± 3.5%	9.1% ± 5.2%
Range	0%-10%	0%-10%	2%-18%

[a] Data as of July 2015

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of TRIM VX[a]

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	1			
Moribund	10	9	4	13
Natural deaths	4	5	10	7
Survivors				
Died last week of study	1			1
Terminal kill	34	36	36	29
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(49)
Periesophageal tissue, inflammation, chronic active	1 (2%)			1 (2%)
Gallbladder	(46)	(46)	(44)	(44)
Inflammation, chronic active	1 (2%)			
Vacuolization cytoplasmic			1 (2%)	
Intestine large, cecum	(50)	(49)	(46)	(45)
Hyperplasia, lymphoid		1 (2%)		
Inflammation	2 (4%)		1 (2%)	1 (2%)
Intestine large, colon	(50)	(50)	(47)	(50)
Hyperplasia, lymphoid				1 (2%)
Inflammation	1 (2%)			
Intestine large, rectum	(48)	(48)	(46)	(45)
Edema	1 (2%)			
Inflammation		1 (2%)		
Intestine small, duodenum	(48)	(46)	(43)	(45)
Hyperplasia, lymphoid		1 (2%)		
Infiltration cellular, lymphoid		1 (2%)		
Inflammation	1 (2%)	3 (7%)	1 (2%)	
Necrosis			1 (2%)	
Intestine small, ileum	(48)	(47)	(44)	(46)
Hyperplasia, lymphoid	4 (8%)	8 (17%)	4 (9%)	2 (4%)
Hyperplasia, plasma cell				1 (2%)
Inflammation	1 (2%)	4 (9%)		
Perforation		1 (2%)		
Serosa, fibrosis		1 (2%)		
Intestine small, jejunum	(48)	(45)	(45)	(46)
Hyperplasia, lymphoid	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Inflammation	1 (2%)	1 (2%)	1 (2%)	
Perforation	1 (2%)			
Ulcer			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	2 (4%)	1 (2%)	
Basophilic focus	6 (12%)	2 (4%)	2 (4%)	4 (8%)
Clear cell focus		5 (10%)	3 (6%)	3 (6%)
Cyst		1 (2%)		
Eosinophilic focus	3 (6%)	8 (16%)	7 (14%)	8 (16%)
Fatty change	1 (2%)	2 (4%)	1 (2%)	
Hematopoietic cell proliferation		1 (2%)	1 (2%)	
Hepatodiaphragmatic nodule		1 (2%)		
Hyperplasia, granulocytic	1 (2%)			
Infarct		1 (2%)		

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Alimentary System (continued)				
Liver (continued)	(50)	(50)	(50)	(50)
Inflammation, chronic active	5 (10%)	1 (2%)	1 (2%)	2 (4%)
Mixed cell focus	1 (2%)	1 (2%)		1 (2%)
Necrosis	1 (2%)	1 (2%)		
Tension lipidosis	1 (2%)	4 (8%)		
Hepatocyte, degeneration				2 (4%)
Hepatocyte, hypertrophy		2 (4%)	1 (2%)	1 (2%)
Hepatocyte, necrosis	3 (6%)	1 (2%)	2 (4%)	5 (10%)
Mesentery	(10)	(10)	(5)	(11)
Inflammation, chronic active	2 (20%)	1 (10%)		
Pigmentation	1 (10%)			
Fat, necrosis	6 (60%)	9 (90%)	5 (100%)	6 (55%)
Pancreas	(50)	(50)	(49)	(50)
Atrophy	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Hypertrophy				3 (6%)
Infiltration cellular, lymphoid	1 (2%)	1 (2%)		1 (2%)
Inflammation, chronic active	3 (6%)		3 (6%)	
Necrosis	1 (2%)			
Duct, cyst	1 (2%)		1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Inflammation, suppurative		1 (2%)		
Necrosis, acute		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Cyst, squamous		1 (2%)		
Hyperkeratosis	2 (4%)	4 (8%)	1 (2%)	2 (4%)
Hyperplasia	3 (6%)	6 (12%)	1 (2%)	2 (4%)
Inflammation			1 (2%)	1 (2%)
Inflammation, chronic active	5 (10%)	1 (2%)	1 (2%)	1 (2%)
Ulcer		1 (2%)	1 (2%)	1 (2%)
Stomach, glandular	(49)	(50)	(49)	(50)
Cyst		1 (2%)	1 (2%)	
Hyperplasia, lymphoid		1 (2%)		
Inflammation, chronic active	4 (8%)	3 (6%)	2 (4%)	1 (2%)
Mineralization	1 (2%)	2 (4%)		
Tooth	(0)	(1)	(0)	(0)
Dysplasia		1 (100%)		
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(49)	(50)
Cardiomyopathy	12 (24%)	18 (36%)	12 (24%)	15 (30%)
Infiltration cellular, lymphoid	1 (2%)			
Inflammation, suppurative			1 (2%)	
Inflammation, chronic active	1 (2%)	5 (10%)	3 (6%)	6 (12%)
Mineralization	1 (2%)		1 (2%)	
Necrosis			2 (4%)	1 (2%)
Thrombosis				1 (2%)
Coronary artery, fibrosis	1 (2%)			
Coronary artery, thrombosis				1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	2 (4%)	2 (4%)	3 (6%)	1 (2%)
Angiectasis		3 (6%)	2 (4%)	2 (4%)
Degeneration, cystic				1 (2%)
Hematopoietic cell proliferation	1 (2%)		1 (2%)	
Hyperplasia	6 (12%)	6 (12%)	2 (4%)	7 (14%)
Hypertrophy	14 (28%)	8 (16%)	13 (26%)	10 (20%)
Inflammation, chronic active	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	11 (22%)	7 (14%)	5 (10%)	5 (10%)
Hypertrophy	1 (2%)		1 (2%)	
Pigmentation				1 (2%)
Islets, pancreatic	(50)	(49)	(49)	(50)
Parathyroid gland	(35)	(30)	(36)	(28)
Ectopic tissue	1 (3%)			
Pituitary gland	(48)	(49)	(49)	(49)
Pars distalis, angiectasis	5 (10%)	2 (4%)	2 (4%)	5 (10%)
Pars distalis, cyst				1 (2%)
Pars distalis, hyperplasia	11 (23%)	17 (35%)	15 (31%)	10 (20%)
Pars distalis, hypertrophy	1 (2%)			1 (2%)
Pars intermedia, hyperplasia	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(49)
Cyst	1 (2%)	1 (2%)	1 (2%)	
Inflammation, suppurative			1 (2%)	
Inflammation, chronic active		2 (4%)		1 (2%)
Follicular cell, hyperplasia	3 (6%)		2 (4%)	2 (4%)
Follicular cell, hypertrophy				1 (2%)
General Body System				
Tissue NOS	(1)	(0)	(0)	(0)
Hemorrhage	1 (100%)			
Genital System				
Clitoral gland	(46)	(48)	(45)	(46)
Ovary	(50)	(49)	(49)	(50)
Angiectasis	1 (2%)	1 (2%)		3 (6%)
Congestion			1 (2%)	
Cyst	13 (26%)	8 (16%)	9 (18%)	7 (14%)
Cyst, multiple	1 (2%)			1 (2%)
Hemorrhage	1 (2%)			
Inflammation, chronic active	2 (4%)	1 (2%)	2 (4%)	
Periovarian tissue, inflammation, chronic active	1 (2%)			
Uterus	(50)	(50)	(50)	(50)
Adenomyosis	1 (2%)			
Angiectasis	1 (2%)	4 (8%)	3 (6%)	2 (4%)
Hemorrhage			1 (2%)	
Inflammation, chronic active	3 (6%)	4 (8%)	3 (6%)	5 (10%)
Necrosis				1 (2%)
Endometrium, hyperplasia, cystic	45 (90%)	35 (70%)	43 (86%)	43 (86%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Atrophy		1 (2%)		
Hyperplasia, granulocytic	1 (2%)			
Myelofibrosis	1 (2%)			
Necrosis				2 (4%)
Myeloid cell, hyperplasia	1 (2%)		4 (8%)	1 (2%)
Lymph node	(11)	(11)	(11)	(10)
Pigmentation			1 (9%)	1 (10%)
Artery, lumbar, inflammation, chronic active				1 (10%)
Artery, renal, inflammation, chronic active				1 (10%)
Iliac, angiectasis			1 (9%)	1 (10%)
Iliac, infiltration cellular, mixed cell	1 (9%)			
Lumbar, angiectasis	1 (9%)	3 (27%)	1 (9%)	1 (10%)
Lumbar, hematopoietic cell proliferation			1 (9%)	
Lumbar, infiltration cellular, plasma cell				1 (10%)
Lumbar, inflammation, suppurative	1 (9%)			
Lumbar, necrosis	1 (9%)			
Pancreatic, hyperplasia, lymphoid		1 (9%)	1 (9%)	
Renal, angiectasis			1 (9%)	1 (10%)
Renal, hyperplasia, lymphoid			1 (9%)	
Renal, infiltration cellular, plasma cell				1 (10%)
Renal, necrosis				1 (10%)
Lymph node, bronchial	(44)	(44)	(44)	(43)
Hematopoietic cell proliferation			1 (2%)	
Hyperplasia, lymphoid	6 (14%)	4 (9%)	9 (20%)	9 (21%)
Infiltration cellular, histiocyte	1 (2%)		2 (5%)	4 (9%)
Inflammation, suppurative			1 (2%)	
Plasma cell, hyperplasia				1 (2%)
Lymph node, mandibular	(35)	(42)	(37)	(39)
Hematopoietic cell proliferation			1 (3%)	
Hyperplasia, lymphoid	4 (11%)	1 (2%)	3 (8%)	1 (3%)
Infiltration cellular, histiocyte			1 (3%)	1 (3%)
Infiltration cellular, plasma cell	1 (3%)			
Lymph node, mediastinal	(40)	(44)	(45)	(45)
Hyperplasia, lymphoid	2 (5%)	4 (9%)	7 (16%)	5 (11%)
Hyperplasia, plasma cell	1 (3%)	1 (2%)		
Infiltration cellular, histiocyte	1 (3%)		1 (2%)	1 (2%)
Pigmentation				1 (2%)
Lymph node, mesenteric	(49)	(49)	(47)	(48)
Angiectasis		3 (6%)	2 (4%)	1 (2%)
Hematopoietic cell proliferation			1 (2%)	1 (2%)
Hyperplasia, lymphoid	11 (22%)	7 (14%)	12 (26%)	4 (8%)
Inflammation, chronic active	1 (2%)			
Necrosis	1 (2%)	1 (2%)		
Artery, inflammation		1 (2%)		
Spleen	(50)	(49)	(48)	(49)
Angiectasis	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Hematopoietic cell proliferation	11 (22%)	7 (14%)	11 (23%)	11 (22%)
Hyperplasia, granulocytic	1 (2%)		1 (2%)	
Hyperplasia, lymphoid	20 (40%)	26 (53%)	17 (35%)	13 (27%)
Inflammation, suppurative	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Necrosis, lymphoid		1 (2%)		1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Hematopoietic System (continued)				
Thymus	(48)	(48)	(47)	(49)
Angiectasis		2 (4%)		3 (6%)
Atrophy	6 (13%)	1 (2%)	7 (15%)	4 (8%)
Cyst	1 (2%)	1 (2%)	1 (2%)	5 (10%)
Ectopic parathyroid gland	2 (4%)	3 (6%)	5 (11%)	5 (10%)
Hyperplasia, lymphoid	11 (23%)	15 (31%)	13 (28%)	11 (22%)
Necrosis, lymphoid				1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Dilatation		1 (2%)		
Hyperplasia	1 (2%)		1 (2%)	1 (2%)
Inflammation	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Edema	1 (2%)		1 (2%)	
Hemorrhage, acute	1 (2%)			
Hyperplasia				1 (2%)
Inflammation, chronic active	2 (4%)	3 (6%)	2 (4%)	5 (10%)
Necrosis				1 (2%)
Ulcer		2 (4%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	6 (12%)	10 (20%)	12 (24%)	9 (18%)
Skeletal muscle	(3)	(4)	(2)	(4)
Degeneration	1 (33%)			
Infiltration cellular, lymphoid	1 (33%)			
Nervous System				
Brain	(50)	(50)	(50)	(50
Compression	5 (10%)	1 (2%)	1 (2%)	3 (6%)
Demyelination			1 (2%)	
Hemorrhage	2 (4%)			
Hydrocephalus	1 (2%)			
Infiltration cellular		1 (2%)	3 (6%)	
Infiltration cellular, lymphocyte		1 (2%)		
Inflammation, chronic active	3 (6%)			
Necrosis, focal		1 (2%)		
Meninges, infiltration cellular			1 (2%)	
Peripheral nerve	(3)	(3)	(1)	(2)
Degeneration	1 (33%)			1 (50%)
Spinal cord	(2)	(3)	(1)	(2)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Inflammation, chronic active	44 (88%)	50 (100%)	48 (96%)	47 (94%)
Necrosis	2 (4%)			3 (6%)
Epiglottis, hyperplasia, squamous	4 (8%)	3 (6%)	16 (32%)	42 (84%)
Epiglottis, metaplasia, squamous		50 (100%)	50 (100%)	50 (100%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m³	30 mg/m³	100 mg/m³
Respiratory System (continued)				
Lung	(50)	(50)	(50)	(50)
Fibrosis			2 (4%)	42 (84%)
Hemorrhage		1 (2%)	2 (4%)	
Hyperplasia, lymphoid				1 (2%)
Infiltration cellular, histiocyte	1 (2%)	4 (8%)	15 (30%)	48 (96%)
Inflammation, chronic	1 (2%)	6 (12%)	26 (52%)	47 (94%)
Metaplasia, osseous				1 (2%)
Metaplasia, squamous				1 (2%)
Mineralization			1 (2%)	
Pigmentation				1 (2%)
Proteinosis			1 (2%)	
Thrombosis	1 (2%)			
Alveolar/bronchiolar epithelium, hyperplasia		3 (6%)	8 (16%)	45 (90%)
Alveolar epithelium, hyperplasia			2 (4%)	43 (86%)
Alveolar epithelium, metaplasia, squamous				1 (2%)
Interstitium, inflammation, suppurative	1 (2%)			
Mediastinum, necrosis			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Cyst, squamous		1 (2%)		
Exudate	8 (16%)	17 (34%)	48 (96%)	49 (98%)
Foreign body		1 (2%)		
Inflammation, chronic active	4 (8%)	25 (50%)	49 (98%)	49 (98%)
Glands, hyperplasia		1 (2%)		
Goblet cell, hyperplasia				2 (4%)
Lateral wall, inflammation, chronic active		2 (4%)		
Nasolacrimal duct, inflammation, chronic active	2 (4%)		2 (4%)	
Nasopharyngeal duct, perforation			14 (28%)	17 (34%)
Olfactory epithelium, accumulation, hyaline droplet	14 (28%)	48 (96%)	50 (100%)	50 (100%)
Olfactory epithelium, atrophy	1 (2%)	2 (4%)	1 (2%)	5 (10%)
Olfactory epithelium, metaplasia, respiratory	2 (4%)	2 (4%)	1 (2%)	4 (8%)
Respiratory epithelium, accumulation, hyaline droplet	23 (46%)	50 (100%)	50 (100%)	50 (100%)
Respiratory epithelium, atrophy	1 (2%)	2 (4%)	28 (56%)	39 (78%)
Respiratory epithelium, hyperplasia	49 (98%)	44 (88%)	48 (96%)	49 (98%)
Respiratory epithelium, metaplasia, squamous	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Respiratory epithelium, necrosis		2 (4%)	13 (26%)	23 (46%)
Turbinate, atrophy			10 (20%)	19 (38%)
Turbinate, perforation			6 (12%)	6 (12%)
Trachea	(49)	(50)	(50)	(49)
Pigmentation				1 (2%)
Cartilage, metaplasia, osseous			2 (4%)	4 (8%)
Epithelium, degeneration				2 (4%)
Epithelium, hyperplasia			1 (2%)	5 (10%)
Epithelium, necrosis				1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Inhalation Study of TRIM VX

	Chamber Control	10 mg/m^3	30 mg/m^3	100 mg/m^3
Special Senses System				
Ear	(0)	(0)	(0)	(1)
Eye	(49)	(50)	(50)	(50)
Inflammation, chronic active			1 (2%)	1 (2%)
Cornea, hyperplasia			1 (2%)	1 (2%)
Harderian gland	(50)	(49)	(49)	(49)
Cyst			1 (2%)	
Hyperplasia	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Hypertrophy		1 (2%)		
Urinary System				
Kidney	(49)	(50)	(49)	(50)
Amyloid deposition			1 (2%)	
Angiectasis				1 (2%)
Cyst	1 (2%)			2 (4%)
Hyperplasia, lymphoid	2 (4%)	4 (8%)		
Infarct, chronic	4 (8%)	5 (10%)	5 (10%)	
Infiltration cellular, lymphoid	1 (2%)			
Inflammation, chronic active	1 (2%)	2 (4%)	1 (2%)	
Metaplasia, osseous		2 (4%)	2 (4%)	
Mineralization	1 (2%)			
Necrosis	1 (2%)			
Nephropathy	38 (78%)	28 (56%)	26 (53%)	29 (58%)
Pigmentation				1 (2%)
Glomerulus, inflammation, acute		1 (2%)		
Renal tubule, hyperplasia	1 (2%)			
Urethra	(0)	(1)	(0)	(0)
Inflammation, chronic active		1 (100%)		
Urinary bladder	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Hyperplasia, lymphoid	2 (4%)	3 (6%)		3 (6%)
Inflammation, chronic active	2 (4%)			

APPENDIX E
GENETIC TOXICOLOGY

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GENETIC TOXICOLOGY

BACTERIAL MUTAGENICITY TEST PROTOCOL

Bacterial mutagenicity assays were conducted according to Zeiger *et al.* (1992), with slight modifications. Samples of TRIM VX were sent to the testing laboratory and coded to ensure that samples were tested blind. TRIM VX was incubated with the *Salmonella typhimurium* tester strains TA98 and TA100 and the *Escherichia coli* strain WP2 *uvrA*/pKM101 either in buffer or 10% S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine or tryptophan (for the *E. coli* strain) and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine- or tryptophan-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of TRIM VX. The high dose was limited by assay design to 10,000 µg/plate. All trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

At the termination of the 3-month toxicity studies with TRIM VX, blood samples (~200 µL) were collected from male and female rats and mice, placed in EDTA-coated tubes, and shipped overnight to the testing laboratory. Upon arrival, blood samples were fixed in ultracold methanol using a MicroFlow^{PLUS} Kit (Litron Laboratories, Rochester, NY) according to the manufacturer's instructions. Fixed samples were stored in a –80° C freezer until analysis. Thawed blood samples were analyzed for frequency of micronucleated reticulocytes (polychromatic erythrocytes, PCEs) and mature erythrocytes (normochromatic erythrocytes, NCEs) using a flow cytometer (Witt *et al*., 2008); both the mature erythrocyte population and the immature reticulocyte population can be analyzed separately by employing special cell surface markers to differentiate the two cell types. Because the very young reticulocyte subpopulation (CD71-positive cells) can be targeted using this technique, rat blood samples can be analyzed for damage that occurred in the bone marrow within the past 24 to 48 hours, before the rat spleen appreciably alters the percentage of micronucleated reticulocytes in circulation (Dertinger *et al*., 2004). In mice, both the reticulocyte and mature erythrocyte populations can be evaluated for micronucleus frequency because the mouse spleen does not sequester and eliminate damaged erythrocytes. These achieve steady state in the peripheral blood of mice following 4 weeks of continuous exposure. Approximately 20,000 reticulocytes and 1×10^6 erythrocytes are analyzed per sample for frequency of micronucleated cells, and the % reticulocytes is calculated as a measure of bone marrow toxicity resulting from chemical exposure.

Based on prior experience with the large number of cells scored using flow cytometric scoring techniques (Kissling *et al*., 2007), it is reasonable to assume that the proportion of micronucleated reticulocytes is approximately normally distributed. The statistical tests selected for trend and for pairwise comparisons with the control group depend on whether the variances among the groups are equal. Levene's test at α=0.05 is used to test for equal variances. In the case of equal variances, linear regression is used to test for a linear trend with dose and Williams' test is used to test for pairwise differences between each treatment group and the control group. In the case of unequal variances, Jonckheere's test is used to test for linear trend and Dunn's test is used for pairwise comparisons of each treatment group with the control group. To correct for multiple pairwise comparisons, the P value for each comparison with the control group is multiplied by the number of comparisons made. In the event that this product is greater than 1.00, it is replaced with 1.00. Trend tests and pairwise comparisons with the controls are considered statistically significant at $P \leq 0.025$.

In the micronucleus test (for each data set: PCEs, NCEs, percentage of PCEs), a positive result is preferably based on the presence of both a significant trend as well as at least one significantly elevated dose group compared with the corresponding control group. The presence of either a significant trend or a single significant dose group generally results in an equivocal call. The absence of both a trend and a significant dose group results in a negative call. Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, reproducibility of any effects observed (in acute studies), and the magnitudes of those effects.

EVALUATION PROTOCOL

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple samples of a chemical were tested in the same assay, and different results were obtained among these samples and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgment of the overall evidence for activity of the chemical in an assay.

RESULTS

TRIM VX (dose range tested, 500 to 10,000 µg/plate) was not mutagenic in *S. typhimurium* strains TA98 or TA100 or in *E. coli* strain WP2 *uvrA*/pKM101 in the presence or absence of exogenous metabolic activation (induced rat liver S9) (Table E1).

In vivo, no increases in the frequencies of micronucleated reticulocytes or erythrocytes were observed in peripheral blood samples from male or female rats or mice exposed to TRIM VX via inhalation (25 to 400 mg/m^3) for three months (Tables E2 and E3).

In addition to the micronucleus endpoint, the percentage of reticulocytes among circulating red blood cells was calculated as a measure of bone marrow toxicity or perturbations in erythropoiesis (Tables E2 and E3). The very small increases in percent reticulocytes noted in male rats and female mice were within historical ranges for this endpoint and, in the absence of any observed hematological effects, were not considered biologically significant.

TABLE E1
Mutagenicity of TRIM VX in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100					
	0	59 ± 7.5	57 ± 1.7	67 ± 8.0	47 ± 0.6
	500	31 ± 5.6	43 ± 2.7	82 ± 3.5	58 ± 3.4
	1,000	31 ± 2.0	41 ± 1.5	55 ± 0.9	55 ± 6.8
	4,000	19 ± 3.2	26 ± 4.0	51 ± 5.2	48 ± 5.2
	7,000	20 ± 3.5	18 ± 2.0	30 ± 1.0	36 ± 1.8
	10,000	50 ± 5.4[c]	24 ± 6.2[c]	30 ± 1.5[c]	30 ± 4.0[c]
Trial summary		Negative	Negative	Negative	Negative
Positive control[b]		354 ± 13.0	459 ± 1.7	637 ± 56.0	598 ± 18.6
TA98					
	0	25 ± 3.8	15 ± 0.7	27 ± 7.0	20 ± 2.3
	500	12 ± 3.6	16 ± 0.9	31 ± 2.1	29 ± 4.1
	1,000	10 ± 1.5	12 ± 1.5	23 ± 1.0	23 ± 2.6
	4,000	13 ± 2.3	14 ± 3.4	31 ± 3.7	25 ± 2.3
	7,000	7 ± 1.2	8 ± 1.9	20 ± 1.2	14 ± 4.0
	10,000	7 ± 0.3[c]	8 ± 2.5[c]	23 ± 8.3[c]	11 ± 0.6[c]
Trial summary		Negative	Negative	Negative	Negative
Positive control		418 ± 17.8	343 ± 52.0	947 ± 41.6	796 ± 63.9
***Escherichia coli* WP2 *uvrA*/pKM101 (analogous to TA102)**					
	0	110 ± 8.5	201 ± 6.8	127 ± 2.6	190 ± 6.7
	500	151 ± 18.6	224 ± 10.1	187 ± 5.2	158 ± 41.6
	1,000	147 ± 5.7	217 ± 4.7	224 ± 6.4	150 ± 29.5
	4,000	122 ± 9.7	177 ± 1.9	185 ± 10.7	191 ± 13.5
	7,000	127 ± 8.5	151 ± 2.3	165 ± 21.7	99 ± 20.0
	10,000	114 ± 9.6[c]	127 ± 4.9[c]	175 ± 16.1[c]	69 ± 5.0[c]
Trial summary		Negative	Negative	Negative	Negative
Positive control		607 ± 24.2	731 ± 27.5	620 ± 68.4	694 ± 64.8

[a] Study was performed at SITEK Research Laboratories. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.

[b] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

[c] Precipitate on plate

TABLE E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Rats Following Treatment with TRIM VX by Inhalation for 3 Months[a]

	Dose (mg/m^3)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[d]	PCEs[b] (%)	P Value[e]
Male								
Air[f]	0	5	0.81 ± 0.08		0.21 ± 0.07		0.775 ± 0.11	
TRIM VX	25	5	0.95 ± 0.08	0.4911	0.11 ± 0.03	1.0000	0.661 ± 0.04	1.0000
	50	5	0.79 ± 0.11	0.5717	0.14 ± 0.02	1.0000	0.817 ± 0.12	0.8235
	100	5	0.74 ± 0.14	0.6049	0.13 ± 0.01	1.0000	0.993 ± 0.12	0.1056
	200	5	0.78 ± 0.10	0.6246	0.19 ± 0.02	0.9229	1.047 ± 0.05	0.0471
	400	5	0.84 ± 0.09	0.5534	0.33 ± 0.11	0.9716	1.014 ± 0.08	0.0479
			P=0.563[g]		P=0.031[h]		P=0.007[g]	
Female								
Air	0	5	0.88 ± 0.19		0.06 ± 0.01		0.851 ± 0.05	
TRIM VX	25	5	0.73 ± 0.15	0.8086	0.05 ± 0.01	0.7388	1.033 ± 0.06	0.6569
	50	5	0.74 ± 0.07	0.8804	0.06 ± 0.01	0.8205	0.924 ± 0.08	1.0000
	100	5	0.77 ± 0.15	0.9046	0.05 ± 0.01	0.8510	1.154 ± 0.13	0.2410
	200	5	0.72 ± 0.09	0.9150	0.05 ± 0.02	0.8644	0.975 ± 0.18	1.0000
	400	5	0.47 ± 0.06	0.9236	0.04 ± 0.01	0.8750	1.182 ± 0.08	0.1297
			P=0.985[g]		P=0.932[g]		P=0.085[h]	

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Witt *et al.* (2008). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the chamber control group; exposed group values are significant at P≤0.025 by William's test.
[d] Pairwise comparison with the chamber control group; exposed group values are significant at P≤0.025 by Dunn's test.
[e] Pairwise comparison with the chamber control group; exposed group values are significant at P≤0.025 by William's test (males) or Dunn's test (females).
[f] Chamber control
[g] Dose-related trend significant at P≤ 0.025 by linear regression
[h] Dose-related trend significant at P≤ 0.025 by Jonckheere's test

TABLE E3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with TRIM VX by Inhalation for 3 Months[a]

	Dose (mg/m^3)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b] (%)	P Value[c]
Male								
Air[d]	0	5	2.46 ± 0.14		1.41 ± 0.04		1.473 ± 0.03	
TRIM VX	25	5	2.28 ± 0.20	0.7182	1.38 ± 0.03	0.6865	1.673 ± 0.07	0.4953
	50	5	2.30 ± 0.16	0.8021	1.40 ± 0.02	0.7720	1.626 ± 0.07	0.5930
	100	5	2.22 ± 0.20	0.8338	1.39 ± 0.04	0.8051	1.522 ± 0.03	0.6348
	200	5	2.48 ± 0.20	0.7655	1.39 ± 0.03	0.8209	1.453 ± 0.06	0.6539
	400	5	2.28 ± 0.14	0.7783	1.37 ± 0.04	0.8337	1.404 ± 0.07	0.4650
			P=0.562[e]		P=0.746		P=0.012	
Female								
Air	0	5	2.21 ± 0.17		1.10 ± 0.04		1.281 ± 0.14	
TRIM VX	25	5	2.04 ± 0.15	0.8089	1.05 ± 0.03	0.9386	1.424 ± 0.08	0.6341
	50	5	1.71 ± 0.13	0.8806	0.99 ± 0.04	0.9717	1.275 ± 0.08	0.7536
	100	5	2.13 ± 0.25	0.9048	1.06 ± 0.02	0.9802	1.401 ± 0.08	0.5874
	200	5	1.83 ± 0.11	0.9152	1.01 ± 0.03	0.9844	1.468 ± 0.21	0.6047
	400	5	2.01 ± 0.21	0.9056	0.97 ± 0.03	0.9868	1.817 ± 0.06	0.0147
			P=0635		P=0.988		P=0.007	

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Witt *et al.* (2008). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the chamber control group; exposed group values are significant at P≤0.025 by William's test.
[d] Chamber control
[e] Dose-related trend significant at P≤0.025 by linear regression

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1 Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of TRIM VX.......... **150**
TABLE F2 Hematology Data for Mice in the 3-Month Inhalation Study of TRIM VX.......... **152**

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of TRIM VX[a]

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
Male						
Hematology						
n	10	10	10	9	10	10
Hematocrit (%)	50.7 ± 0.5	49.9 ± 0.7	50.3 ± 0.6	49.4 ± 0.6[b]	50.8 ± 0.4	49.3 ± 0.4
Packed cell volume (%)	51.5 ± 0.5	51.3 ± 0.7	51.0 ± 0.7	50.2 ± 0.6	52.1 ± 0.7	50.7 ± 0.6
Hemoglobin (g/dL)	16.4 ± 0.1	16.3 ± 0.2	16.3 ± 0.2	16.1 ± 0.2	16.5 ± 0.2	16.1 ± 0.2
Erythrocytes (10^6/µL)	9.22 ± 0.09	9.30 ± 0.17	9.19 ± 0.19	9.12 ± 0.11	9.31 ± 0.11	9.12 ± 0.11
Reticulocytes (10^3/µL)	213.7 ± 12.3	198.2 ± 7.2	209.0 ± 9.0	214.0 ± 6.5	212.8 ± 8.4	219.5 ± 10.0
Nucleated erythrocytes/ 100 leukocytes	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean cell volume (fL)	55.9 ± 0.5	55.2 ± 0.6	55.6 ± 0.8	55.1 ± 0.5	56.0 ± 0.5	55.6 ± 0.7
Mean cell hemoglobin (pg)	17.8 ± 0.2	17.6 ± 0.2	17.8 ± 0.3	17.7 ± 0.2	17.7 ± 0.2	17.6 ± 0.2
Mean cell hemoglobin concentration (g/dL)	31.9 ± 0.3	31.8 ± 0.2	32.0 ± 0.3	32.1 ± 0.3	31.6 ± 0.3	31.7 ± 0.2
Platelets (10^3/µL)	740 ± 23	756 ± 56	780 ± 25	800 ± 52	836 ± 42	800 ± 34
Leukocytes (10^3/µL)	8.65 ± 0.61	8.42 ± 0.34	9.09 ± 0.54	8.52 ± 0.23	8.76 ± 0.38	8.67 ± 0.55
Segmented neutrophils (10^3/µL)	1.23 ± 0.11	1.14 ± 0.10	1.21 ± 0.11	1.09 ± 0.08	1.20 ± 0.08	1.26 ± 0.10
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	7.17 ± 0.59	7.02 ± 0.27	7.57 ± 0.44	7.19 ± 0.16	7.25 ± 0.36	7.11 ± 0.54
Monocytes (10^3/µL)	0.15 ± 0.02	0.15 ± 0.01	0.19 ± 0.02	0.15 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
Basophils (10^3/µL)	0.02 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Eosinophils (10^3/µL)	0.08 ± 0.00	0.09 ± 0.02	0.09 ± 0.02	0.08 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)	15.3 ± 0.7	17.8 ± 0.4	17.5 ± 0.7	17.1 ± 1.0	16.1 ± 0.6	18.1 ± 0.6*
Creatinine (mg/dL)	0.41 ± 0.01	0.44 ± 0.02	0.41 ± 0.02	0.47 ± 0.02*	0.46 ± 0.02*	0.47 ± 0.02*
Glucose (mg/dL)	132 ± 10	127 ± 4	125 ± 3	140 ± 8	139 ± 13	132 ± 5
Total protein (g/dL)	7.1 ± 0.1	7.0 ± 0.1	7.1 ± 0.0	7.0 ± 0.1	7.1 ± 0.1	7.0 ± 0.1
Albumin (g/dL)	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.0	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.1
Globulin (g/dL)	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.4 ± 0.1
Albumin/globulin ratio	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.0	1.9 ± 0.1
Cholesterol (mg/dL)	83 ± 4	82 ± 5	82 ± 3	78 ± 3	82 ± 5	81 ± 5
Triglycerides (mg/dL)	97 ± 13	97 ± 8	112 ± 11	86 ± 12	95 ± 11	87 ± 10
Alanine aminotransferase (IU/L)	34 ± 1	34 ± 2	34 ± 1	38 ± 5	33 ± 2	40 ± 6
Alkaline phosphatase (IU/L)	107 ± 6	123 ± 8	129 ± 11	114 ± 11	120 ± 7	119 ± 9
Creatine kinase (IU/L)	151 ± 18	167 ± 22	156 ± 15	332 ± 118	152 ± 21	487 ± 200*
Sorbitol dehydrogenase (IU/L)	12 ± 1	11 ± 1	12 ± 1	9 ± 1	11 ± 1	11 ± 1
Bile acids (µmol/L)	5.9 ± 1.3	10.8 ± 3.2	10.6 ± 3.9	17.3 ± 4.6	9.4 ± 2.8	6.5 ± 2.8

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Inhalation Study of TRIM VX

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
Female						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)	48.2 ± 0.7	47.1 ± 0.5	47.9 ± 0.4	47.3 ± 0.5	47.1 ± 0.4	46.5 ± 0.7
Packed cell volume (%)	49.4 ± 0.9	48.2 ± 0.6	49.1 ± 0.4	48.2 ± 0.5	47.8 ± 0.4	47.6 ± 0.6
Hemoglobin (g/dL)	16.1 ± 0.2	15.6 ± 0.2	15.9 ± 0.2	15.5 ± 0.2	15.6 ± 0.1	15.5 ± 0.1
Erythrocytes (10^6/µL)	8.55 ± 0.13	8.29 ± 0.11	8.51 ± 0.10	8.24 ± 0.07	8.34 ± 0.10	8.24 ± 0.11
Reticulocytes (10^3/µL)	229.4 ± 14.1	246.8 ± 6.2	245.7 ± 13.1	240.8 ± 8.4	247.4 ± 9.8	248.0 ± 10.0
Nucleated erythrocytes/ 100 leukocytes	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean cell volume (fL)	57.8 ± 0.6	58.2 ± 0.3	57.7 ± 0.5	58.6 ± 0.4	57.4 ± 0.5	57.7 ± 0.4
Mean cell hemoglobin (pg)	18.8 ± 0.1	18.9 ± 0.1	18.7 ± 0.2	18.9 ± 0.2	18.7 ± 0.2	18.8 ± 0.2
Mean cell hemoglobin concentration (g/dL)	32.5 ± 0.2	32.4 ± 0.1	32.4 ± 0.3	32.2 ± 0.1	32.6 ± 0.2	32.6 ± 0.2
Platelets (10^3/µL)	720 ± 32	690 ± 33	691 ± 19	773 ± 28	788 ± 34	751 ± 41
Leukocytes (10^3/µL)	6.25 ± 0.55	6.52 ± 0.29	6.38 ± 0.40	5.85 ± 0.67	6.58 ± 0.47	5.92 ± 0.48
Segmented neutrophils (10^3/µL)	1.06 ± 0.12	1.07 ± 0.11	1.11 ± 0.10	0.86 ± 0.09	1.08 ± 0.12	0.90 ± 0.07
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	4.97 ± 0.44	5.22 ± 0.23	5.04 ± 0.38	4.79 ± 0.58	5.23 ± 0.44	4.76 ± 0.45
Monocytes (10^3/µL)	0.14 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.16 ± 0.02	0.12 ± 0.02
Basophils (10^3/µL)	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00**	0.03 ± 0.00*
Eosinophils (10^3/µL)	0.06 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.11 ± 0.01*
Clinical Chemistry						
Urea nitrogen (mg/dL)	18.3 ± 0.5	20.1 ± 1.0	21.1 ± 1.7	22.8 ± 2.6	25.5 ± 4.0	19.0 ± 1.0
Creatinine (mg/dL)	0.46 ± 0.03	0.42 ± 0.01	0.40 ± 0.01	0.43 ± 0.02	0.43 ± 0.02	0.45 ± 0.02
Glucose (mg/dL)	145 ± 5	123 ± 4*	132 ± 4	135 ± 4	136 ± 6	145 ± 9
Total protein (g/dL)	7.4 ± 0.1	7.6 ± 0.1	7.5 ± 0.1	7.5 ± 0.1	7.5 ± 0.1	7.4 ± 0.1
Albumin (g/dL)	5.4 ± 0.1	5.5 ± 0.1	5.4 ± 0.1	5.3 ± 0.1	5.2 ± 0.1	5.3 ± 0.1
Globulin (g/dL)	2.0 ± 0.1	2.1 ± 0.0	2.1 ± 0.1	2.2 ± 0.1	2.2 ± 0.1*	2.2 ± 0.1
Albumin/globulin ratio	2.7 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.4 ± 0.1*	2.3 ± 0.1**	2.5 ± 0.1*
Cholesterol (mg/dL)	62 ± 3	62 ± 3	57 ± 3	62 ± 3	63 ± 4	61 ± 2
Triglycerides (mg/dL)	54 ± 5	73 ± 8	56 ± 4	64 ± 4	76 ± 7*	62 ± 6
Alanine aminotransferase (IU/L)	30 ± 1	34 ± 4	31 ± 2	30 ± 1	30 ± 2	31 ± 2
Alkaline phosphatase (IU/L)	75 ± 7	82 ± 11	73 ± 6	77 ± 6	83 ± 8	54 ± 3
Creatine kinase (IU/L)	208 ± 34	197 ± 22	189 ± 22	233 ± 48	177 ± 24	289 ± 65
Sorbitol dehydrogenase (IU/L)	12 ± 1	12 ± 1	12 ± 1	11 ± 1	11 ± 1	8 ± 1*
Bile acids (µmol/L)	11.8 ± 3.6	8.4 ± 2.6	7.9 ± 1.5	10.6 ± 3.1	7.4 ± 2.1	13.7 ± 3.7

* Significantly different (P≤0.05) from the chamber control group by Dunn's or Shirley's test
** Significantly different (P≤0.01) from the chamber control group by Shirley's test
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=8

TABLE F2
Hematology Data for Mice in the 3-Month Inhalation Study of TRIM VX[a]

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
n	10	10	10	10	10	10
Male						
Hematocrit (%)	51.8 ± 0.6	51.8 ± 0.3	51.8 ± 0.7	51.7 ± 0.3	49.0 ± 1.1*	51.0 ± 0.5
Packed cell volume (%)	53.5 ± 0.7	52.8 ± 0.3	52.3 ± 0.5	53.1 ± 0.4	50.3 ± 1.1**	51.7 ± 0.4**
Hemoglobin (g/dL)	17.1 ± 0.2	16.8 ± 0.1	16.7 ± 0.1	17.0 ± 0.1	16.1 ± 0.3**	16.6 ± 0.1
Erythrocytes (10^6/µL)	11.09 ± 0.13	10.93 ± 0.06	10.80 ± 0.08	10.96 ± 0.07	10.44 ± 0.24**	10.64 ± 0.09**
Reticulocytes (10^3/µL)	306.5 ± 5.0	306.3 ± 5.9	287.5 ± 15.7	297.8 ± 7.2	270.9 ± 13.2**	270.9 ± 7.9**
Nucleated erythrocytes/ 100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Howell-Jolly bodies (% erythrocytes)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Mean cell volume (fL)	48.2 ± 0.2	48.3 ± 0.2	48.5 ± 0.4	48.4 ± 0.2	48.2 ± 0.3	48.6 ± 0.2
Mean cell hemoglobin (pg)	15.4 ± 0.0	15.4 ± 0.1	15.5 ± 0.1	15.5 ± 0.0	15.5 ± 0.1	15.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)	31.9 ± 0.2	31.8 ± 0.1	32.0 ± 0.1	32.0 ± 0.1	32.1 ± 0.2	32.1 ± 0.1
Platelets (10^3/µL)	1,232 ± 53	1,384 ± 23	1,323 ± 25	1,275 ± 49	1,426 ± 89	1,395 ± 43
Leukocytes (10^3/µL)	3.25 ± 0.34	3.06 ± 0.45	3.09 ± 0.25	3.33 ± 0.35	3.00 ± 0.55	2.77 ± 0.26
Segmented neutrophils (10^3/µL)	0.40 ± 0.06	0.37 ± 0.06	0.42 ± 0.06	0.46 ± 0.08	0.48 ± 0.14	0.31 ± 0.04
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	2.70 ± 0.28	2.55 ± 0.38	2.53 ± 0.19	2.76 ± 0.27	2.38 ± 0.42	2.34 ± 0.21
Monocytes (10^3/µL)	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.00	0.02 ± 0.01	0.05 ± 0.02	0.02 ± 0.01
Basophils (10^3/µL)	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
Eosinophils (10^3/µL)	0.10 ± 0.02	0.10 ± 0.02	0.10 ± 0.01	0.09 ± 0.03	0.08 ± 0.02	0.09 ± 0.02
Female						
Hematocrit (%)	50.8 ± 0.4	50.9 ± 0.3	51.1 ± 0.5	50.5 ± 0.4	49.7 ± 0.4	50.1 ± 0.3
Packed cell volume (%)	51.4 ± 0.4	51.2 ± 0.4	51.9 ± 0.5	50.7 ± 0.4	50.4 ± 0.5	50.5 ± 0.4
Hemoglobin (g/dL)	16.9 ± 0.1	16.8 ± 0.1	16.9 ± 0.2	16.7 ± 0.1	16.7 ± 0.1	16.6 ± 0.1
Erythrocytes (10^6/µL)	10.66 ± 0.08	10.57 ± 0.08	10.78 ± 0.10	10.60 ± 0.09	10.50 ± 0.09	10.48 ± 0.06
Reticulocytes (10^3/µL)	305.1 ± 12.8	315.7 ± 15.8	287.3 ± 9.0	269.0 ± 7.9	293.1 ± 19.0	322.4 ± 16.9
Nucleated erythrocytes/ 100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Howell-Jolly bodies (% erythrocytes)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Mean cell volume (fL)	48.3 ± 0.2	48.5 ± 0.2	48.1 ± 0.2	47.9 ± 0.1	48.0 ± 0.1	48.1 ± 0.1
Mean cell hemoglobin (pg)	15.9 ± 0.1	15.9 ± 0.1	15.7 ± 0.1	15.8 ± 0.1	15.9 ± 0.1	15.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.8 ± 0.1	32.7 ± 0.2	32.6 ± 0.2	32.9 ± 0.1	33.0 ± 0.2	33.0 ± 0.1
Platelets (10^3/µL)	1,178 ± 39	1,153 ± 36	1,092 ± 29	1,222 ± 20	1,174 ± 35	1,216 ± 35
Leukocytes (10^3/µL)	2.74 ± 0.22	2.70 ± 0.27	2.90 ± 0.21	3.26 ± 0.21	3.28 ± 0.35	3.08 ± 0.17
Segmented neutrophils (10^3/µL)	0.36 ± 0.03	0.30 ± 0.05	0.30 ± 0.04	0.41 ± 0.05	0.34 ± 0.06	0.34 ± 0.06
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	2.26 ± 0.18	2.26 ± 0.21	2.47 ± 0.19	2.70 ± 0.17	2.84 ± 0.29	2.63 ± 0.16
Monocytes (10^3/µL)	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.00
Basophils (10^3/µL)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00**	0.01 ± 0.00*
Eosinophils (10^3/µL)	0.08 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.10 ± 0.02	0.07 ± 0.02	0.07 ± 0.02

* Significantly different (P≤0.05) from the chamber control group by Dunn's or Shirley's test
** P≤0.01
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

APPENDIX G
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE G1 **Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Inhalation Study of TRIM VX**............154
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TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Inhalation Study of TRIM VX[a]

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
n	10	10	10	10	10	10
Male						
Necropsy body wt	425 ± 8	387 ± 8*	416 ± 9	404 ± 8	413 ± 12	380 ± 14*
Heart						
Absolute	1.12 ± 0.03	1.03 ± 0.03	1.16 ± 0.04	1.10 ± 0.03	1.13 ± 0.03	1.08 ± 0.04
Relative	2.644 ± 0.058	2.661 ± 0.047	2.782 ± 0.030	2.730 ± 0.043	2.755 ± 0.062	2.857 ± 0.084*
R. Kidney						
Absolute	1.29 ± 0.02	1.19 ± 0.02	1.33 ± 0.04	1.32 ± 0.04	1.34 ± 0.05	1.27 ± 0.05
Relative	3.027 ± 0.043	3.085 ± 0.058	3.193 ± 0.052	3.267 ± 0.067*	3.242 ± 0.084*	3.342 ± 0.069**
Liver						
Absolute	12.30 ± 0.35	11.75 ± 0.25	12.52 ± 0.30	12.18 ± 0.47	12.87 ± 0.39	12.59 ± 0.73
Relative	28.903 ± 0.499	30.416 ± 0.370	30.125 ± 0.452	30.101 ± 0.785	31.244 ± 0.645*	33.002 ± 0.935**
Lung						
Absolute	2.50 ± 0.13	2.17 ± 0.11	2.44 ± 0.16	2.44 ± 0.14	2.52 ± 0.08	2.54 ± 0.12
Relative	5.893 ± 0.311	5.622 ± 0.294	5.890 ± 0.430	6.048 ± 0.312	6.154 ± 0.250	6.715 ± 0.334
Spleen						
Absolute	0.665 ± 0.025	0.607 ± 0.035	0.705 ± 0.021	0.665 ± 0.021	0.689 ± 0.023	0.651 ± 0.022
Relative	1.564 ± 0.054	1.575 ± 0.094	1.702 ± 0.060	1.649 ± 0.046	1.674 ± 0.047	1.725 ± 0.066
R. Testis						
Absolute	1.847 ± 0.064	1.833 ± 0.059	1.841 ± 0.036	1.838 ± 0.051	1.909 ± 0.043	1.720 ± 0.049
Relative	4.361 ± 0.180	4.749 ± 0.145	4.434 ± 0.078	4.562 ± 0.131	4.661 ± 0.170	4.558 ± 0.149
Thymus						
Absolute	0.519 ± 0.024	0.517 ± 0.038	0.524 ± 0.028	0.507 ± 0.025	0.526 ± 0.036	0.513 ± 0.023
Relative	1.222 ± 0.058	1.333 ± 0.084	1.262 ± 0.069	1.254 ± 0.054	1.265 ± 0.066	1.369 ± 0.089
Female						
Necropsy body wt	237 ± 6	222 ± 5	230 ± 5	232 ± 6	236 ± 6	227 ± 6
Heart						
Absolute	0.76 ± 0.02	0.73 ± 0.02	0.75 ± 0.01	0.78 ± 0.02	0.79 ± 0.01	0.76 ± 0.02
Relative	3.222 ± 0.059	3.311 ± 0.035	3.283 ± 0.053	3.353 ± 0.069	3.349 ± 0.061	3.372 ± 0.049
R. Kidney						
Absolute	0.83 ± 0.02	0.81 ± 0.03	0.82 ± 0.03	0.81 ± 0.02	0.87 ± 0.03	0.84 ± 0.02
Relative	3.509 ± 0.094	3.644 ± 0.049	3.550 ± 0.085	3.515 ± 0.091	3.708 ± 0.116	3.721 ± 0.071
Liver						
Absolute	7.39 ± 0.21	6.95 ± 0.25	7.14 ± 0.25	7.56 ± 0.15	8.25 ± 0.36*	8.37 ± 0.25**
Relative	31.311 ± 0.855	31.326 ± 0.758	31.045 ± 0.450	32.658 ± 0.620	34.940 ± 0.888**	36.843 ± 0.390**
Lung						
Absolute	1.60 ± 0.08	1.40 ± 0.06	1.56 ± 0.07	1.85 ± 0.11*	1.86 ± 0.08*	1.85 ± 0.06*
Relative	6.779 ± 0.279	6.301 ± 0.182	6.778 ± 0.199	7.930 ± 0.368**	7.953 ± 0.378**	8.157 ± 0.246**
Spleen						
Absolute	0.459 ± 0.029	0.406 ± 0.023	0.415 ± 0.015	0.441 ± 0.014	0.464 ± 0.019	0.480 ± 0.020
Relative	1.943 ± 0.120	1.823 ± 0.072	1.821 ± 0.090	1.914 ± 0.081	1.990 ± 0.110	2.123 ± 0.096
Thymus						
Absolute	0.434 ± 0.021	0.431 ± 0.015	0.452 ± 0.021	0.463 ± 0.016	0.454 ± 0.032	0.455 ± 0.024
Relative	1.834 ± 0.083	1.948 ± 0.059	1.980 ± 0.104	1.998 ± 0.052	1.922 ± 0.106	2.016 ± 0.116

* Significantly different ($P \le 0.05$) from the chamber control group by Williams' or Dunnett's test
** Significantly different ($P \le 0.01$) from the chamber control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Inhalation Study of TRIM VX[a]

	Chamber Control	25 mg/m^3	50 mg/m^3	100 mg/m^3	200 mg/m^3	400 mg/m^3
n	10	10	10	10	10	10
Male						
Necropsy body wt	37.6 ± 0.8	35.8 ± 0.4	37.0 ± 0.7	36.1 ± 0.8	36.4 ± 0.6	34.7 ± 0.6*
Heart						
Absolute	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.16 ± 0.00
Relative	4.311 ± 0.131	4.493 ± 0.127	4.461 ± 0.127	4.498 ± 0.112	4.714 ± 0.166	4.468 ± 0.106
R. Kidney						
Absolute	0.31 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.29 ± 0.00
Relative	8.315 ± 0.137	8.737 ± 0.165	8.367 ± 0.142	8.659 ± 0.104	8.523 ± 0.114	8.455 ± 0.148
Liver						
Absolute	1.54 ± 0.05	1.58 ± 0.03	1.71 ± 0.05	1.64 ± 0.04	1.81 ± 0.07**	1.76 ± 0.05**
Relative	40.795 ± 0.675	43.982 ± 0.513*	46.136 ± 1.159**	45.386 ± 0.861**	49.719 ± 1.359**	50.636 ± 0.900**
Lung						
Absolute	0.23 ± 0.02	0.21 ± 0.00	0.23 ± 0.01	0.25 ± 0.01	0.29 ± 0.01**	0.30 ± 0.00**
Relative	6.044 ± 0.420	5.862 ± 0.113	6.188 ± 0.189	6.886 ± 0.167*	7.851 ± 0.162**	8.684 ± 0.135**
Spleen						
Absolute	0.061 ± 0.002	0.065 ± 0.002	0.072 ± 0.003**	0.070 ± 0.003*	0.077 ± 0.004**	0.068 ± 0.002**
Relative	1.621 ± 0.046	1.815 ± 0.049	1.948 ± 0.070**	1.949 ± 0.085**	2.120 ± 0.106**	1.961 ± 0.057**
R. Testis						
Absolute	0.111 ± 0.004	0.112 ± 0.002	0.115 ± 0.001	0.111 ± 0.002	0.110 ± 0.002	0.114 ± 0.001
Relative	2.955 ± 0.094	3.126 ± 0.062	3.121 ± 0.057	3.096 ± 0.056	3.012 ± 0.052	3.294 ± 0.075**
Thymus						
Absolute	0.049 ± 0.003	0.047 ± 0.002	0.047 ± 0.002	0.042 ± 0.002	0.043 ± 0.001	0.049 ± 0.002
Relative	1.298 ± 0.071	1.310 ± 0.060	1.264 ± 0.042	1.161 ± 0.050	1.182 ± 0.037	1.412 ± 0.068
Female						
Necropsy body wt	29.8 ± 0.9	31.0 ± 0.9	30.7 ± 1.1	29.1 ± 0.8	30.1 ± 0.6	29.2 ± 0.5
Heart						
Absolute	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00
Relative	4.659 ± 0.136	4.566 ± 0.128	4.679 ± 0.152	4.731 ± 0.110	4.697 ± 0.107	4.765 ± 0.097
R. Kidney						
Absolute	0.21 ± 0.01	0.21 ± 0.00	0.21 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.21 ± 0.00
Relative	7.012 ± 0.158	6.765 ± 0.151	6.940 ± 0.183	7.157 ± 0.228	7.187 ± 0.178	7.134 ± 0.189
Liver						
Absolute	1.35 ± 0.04	1.50 ± 0.06	1.51 ± 0.06	1.37 ± 0.04	1.57 ± 0.02**	1.70 ± 0.04**
Relative	45.360 ± 1.019	48.331 ± 0.916	49.196 ± 1.141	46.971 ± 1.036	52.298 ± 1.057**	58.078 ± 1.027**
Lung						
Absolute	0.21 ± 0.00	0.22 ± 0.01	0.22 ± 0.01	0.25 ± 0.01**	0.27 ± 0.01**	0.30 ± 0.01**
Relative	6.973 ± 0.262	7.103 ± 0.122	7.284 ± 0.255	8.519 ± 0.276**	9.123 ± 0.256**	10.382 ± 0.181**
Spleen						
Absolute	0.093 ± 0.003	0.096 ± 0.005	0.097 ± 0.004	0.089 ± 0.003	0.102 ± 0.003	0.106 ± 0.003
Relative	3.129 ± 0.088	3.090 ± 0.102	3.167 ± 0.113	3.057 ± 0.077	3.391 ± 0.095	3.634 ± 0.098**
Thymus						
Absolute	0.053 ± 0.004	0.059 ± 0.003	0.060 ± 0.004	0.059 ± 0.002	0.054 ± 0.003	0.051 ± 0.003
Relative	1.772 ± 0.149	1.914 ± 0.087	1.940 ± 0.120	2.013 ± 0.049	1.778 ± 0.077	1.737 ± 0.096

* Significantly different ($P \leq 0.05$) from the chamber control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

APPENDIX H
CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

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CHEMICAL CHARACTERIZATION AND GENERATION OF CHAMBER CONCENTRATIONS

PROCUREMENT AND CHARACTERIZATION OF TRIM® VX

TRIM VX was obtained from Master Chemical Corporation (Perrysburg, OH) in two lots (101607N and 011509N). Lot 101607N was used during the 3-month studies, and lot 011509N was used during the 2-year studies. Characterization and stability analyses of the test article were conducted by the analytical chemistry laboratory at Chemir Analytical Services (Maryland Heights, MO) and by the study laboratory at Battelle Toxicology Northwest (Richland, WA). Reports on analyses performed in support of the TRIM VX studies are on file at the National Institute of Environmental Health Sciences.

The test article was a dark brown liquid. Fourier transform infrared (FTIR) spectroscopy was used to estimate the relative presence of various functional groups and create a reference for future comparisons of the same lot. A representative FTIR spectrum is presented in Figure H1.

Analyses for both lots performed by the analytical chemistry laboratory included Karl Fischer titration for water content; elemental analysis for carbon, hydrogen, nitrogen, and sulfur using a C, H, N, S analyzer; metal analysis using inductively coupled plasma/optical emission spectrometry (ICP/OES); chlorine, chloride, nitrate, and nitrite analysis by ion chromatography (IC) with conductivity detection; and iodine and iodide by ion-selective electrode (ISE) titration. Analyses for both lots performed by the study laboratory included industry-standard determinations of specific gravity, pH, and refractive index; determination of total n-hexane extractable material; determination of bacteria and fungi; initial identification of general organic components using gas chromatography (GC) with flame ionization detection (FID) by system A (Table H1), and the identification of the major components using GC with mass spectrometry (MS) detection by system B (lot 101607N only); determination of alkanolamines using liquid chromatography (LC)/MS as described below; and quantitation of major oil constituents using GC/FID by systems C and D (lot 011509N only). Samples were collected from the top, middle, and bottom of the drum and analyzed in triplicate to determine the specific gravity, pH, refractive index, bacteria and fungi, total n-hexane extractable material, and organic constituents.

The total amount of n-hexane extractable material was determined using United States Environmental Protection Agency Method #1664 (USEPA, 1999), and the amounts of bacteria and fungi were determined using a Sani-Check® test kit (Biosan Laboratories, Warren, MI); samples were diluted to 10% with sterile water and applied to paddles coated on one side with media for the determination of bacteria and on the other side for the determination of fungi, then incubated for 7 days at 25° to 30° C. The identification and quantitation of alkanolamines were determined using LC/MS: the system included an Agilent 6410 Triple Quad LC/MS instrument (Agilent Technologies, Inc., Santa Clara, CA), a Waters Nova-Pak® C_{18} column (300 mm × 3.9 mm, 4 µm particle size) (Waters Corporation, Milford, MA), an isocratic mobile phase of 0.1% formic acid in water, and a flow rate of 0.7 mL/minute.

For lot 101607N, the water content was 7.1%, pH was 7.5, specific gravity was 1.003, and refractive index was 1.485. Elemental analysis indicated 67.5% carbon, 10.8% hydrogen, less than 0.5% nitrogen, and 1.6% sulfur; metal analysis by ICP/OES indicated all elements were below the level of detection; IC results indicated 8.6% chlorine, 187 ppm chloride, and nitrate and nitrite less than 18 ppm; and ISE titration indicated 12 ppm iodine and less than 5 ppm iodide. GC/FID analysis for general organic components indicated 12 separate peaks. Using standard addition and GC/MS, the peaks were identified as propylene glycol, diethylene glycol, diethylene glycol monobutyl ether, α-terpineol, 4-chloro-3-methyl-phenol, triethanolamine, myristic acid, methyl palmitate, palmitic acid, methyl oleate, methyl stearate, and oleic acid. Triethanolamine was determined to be present at 3.7% by LC/MS. Quantitation of 11 compounds in the oil fraction (Table H2) was obtained using a second GC/FID system and indicated 1.18% methyl palmitate, 5.65% methyl oleate, and 0.89% methyl stearate for the fatty acid methyl esters. The total amount of n-hexane extractable material was determined to be approximately 80.2% by weight. The amount of bacteria was less than 100 colony forming units (CFU)/mL and the amount of fungi was less than 10 CFU/mL.

For lot 011509N, the water content was 6.8%, pH was 7.6, specific gravity was 1.008, and refractive index was 1.485. Elemental analysis indicated 65.4% carbon, 10.5% hydrogen, less than 0.5% nitrogen, and 1.8% sulfur; metal analysis by ICP/OES indicated all elements were below the level of detection; IC results indicated 8.8% chlorine, 249 ppm chloride, and nitrate and nitrite each less than 40 ppm; and ISE titration indicated less than 9 ppm iodine and less than 5 ppm iodide. GC/FID analysis for general organic components indicated nine separate peaks. Using the earlier standard addition and GC/MS analysis of lot 101607N, the peaks were identified as propylene glycol, diethylene glycol, diethylene glycol monobutyl ether, α-terpineol, 4-chloro-3-methyl-phenol, triethanolamine, methyl palmitate, methyl oleate, and methyl stearate. Triethanolamine was determined to be present at 3.2% by LC/MS. Quantitation of eight compounds in the oil fraction (Table H2) was obtained using a second GC/FID system and indicated 1.20% methyl palmitate, 5.81% methyl oleate, and 0.93% methyl stearate for the fatty acid methyl esters. Using GC/FID and an alternate analytical column, myristic, oleic, and palmitic fatty acids were quantitated at 0.23%, 1.23%, and 0.31%, respectively. The total amount of n-hexane extractable material was determined to be approximately 85% by weight. The amount of bacteria was less than 100 CFU/mL and the amount of fungi was less than 10 CFU/mL.

The test article was determined to be composed of water, alkanolamines, and oil. The pH of the test article was approximately 7.5. In general, the FTIR spectra were consistent with the presence of organic amines. The test article did not contain significant amounts of water soluble nitrates, nitrites, chlorides, or iodides. The mass balance percentages based on elemental contributions resulted in 95% (lot 101607N) and 93% (lot 011509N) coverage. There were no differences between the two lots.

Periodic reanalyses of lot 011509N were performed by the analytical chemistry laboratory and the study laboratory at least every 6 months during the 2-year studies. FTIR spectra were obtained and compared to the reference spectrum of this lot; determinations were made for the pH, specific gravity, and refractive index; determination and quantitation of alkanolamines was performed using LC/MS as described above; assessment of fatty acid methyl esters was performed using GC/FID by system C (Table H1); and the amounts of bacteria and fungi were determined using a Sani-Check® BF test kit. To ensure stability, the bulk test article was stored at room temperature, protected from light in metal drums, and no degradation of the bulk test article was detected based on these analyses (Table H3).

Following completion of the 2-year study, pH of bulk lot 011509N was measured as neat and 10% solutions using a VWR Symphony 14002-784 Posi-PHlo liquid-filled flushable-junction combination electrode with epoxy body (similar to the one used during the bioassay) and Thermo Scientific Orion 912600 low-maintenance sealed gel-filled electrode with epoxy body. The pH values of the neat and 10% solutions, respectively, were 7.56 and 8.68 when the liquid-filled electrode was used and 8.20 and 8.69 when the gel-filled electrode was used. The pH of the 10% solutions was similar (8.68 to 8.69) regardless of the type of probe used and was within the range (8.3 to 9.3) listed in the manufacturer's data sheet for TRIM VX.

AEROSOL GENERATION AND EXPOSURE SYSTEM

The generation and delivery system used in the 3-month and 2-year studies consisted of two generator assemblies configured together so that the output from each assembly was directed to a common distribution line (Figure H2). For the 2-year studies, male and female rats were housed in separate chambers; female rat chambers also housed male and female mice from the concurrent study. Each assembly contained three multi-jet nebulizers, of which one was operational and two were backups. The bottom of the generator assembly contained the liquid reservoir. TRIM VX was continuously pumped to the liquid reservoir from the chemical cabinet reservoir by metering pumps during the aerosol generation process to ensure a fresh supply of test article and pumping rates that exceeded the rates at which aerosol was removed from the generator assemblies. Ports in the generator assembly introduced compressed air to drive the nebulizers and dilution air to transport aerosol to the distribution line.

Each nebulizer assembly consisted of a multi-jet thimble nebulizer, a liquid uptake tube, and a compressed air supply port. High velocity compressed air created a vacuum in the liquid uptake tube that drew test article from the liquid reservoir into the multi-jet nebulizer streams where shear forces broke the resultant liquid filaments into droplets. Large droplets were impacted on the impaction plate of the nebulizer or the generator assembly walls and were returned to the liquid reservoir. Smaller droplets were drawn into the dilution air and transported to the common distribution line made of bonded stainless steel, grounded to prevent electrostatic charge buildup. The common distribution line was divided into two branches to supply aerosol to exposure chambers located on both sides of the exposure room; each branch line terminated in a filter protecting the flowmeter controlling the line via the house vacuum supply. A second distribution line flow control system used during nonexposure periods consisted of a HEPA filter protecting the airvac pump that created a vacuum in the distribution lines that exceeded the pressures in the chambers, creating a minimal backflow from each chamber inlet tee ensuring that the test article did not migrate into the chambers during off exposure periods.

During exposures, at each chamber position, aerosol was removed from the distribution line and injected into a tee fitting where it was directed either to the inlet of the exposure chamber where it was mixed with conditioned air or to siphon flow exhaust. Conditioned air was defined as the mix of air derived from each exposure chamber's wet and dry air duct supplies. The temperature of the resultant mixture of air was adjusted by passage over a temperature-controlled radiator after sequential treatment with Purafil, charcoal, and HEPA filters. Target dewpoint temperatures of the wet and dry ducts were 60° F and 40° F, respectively. Air for the ducts was obtained from the building air supply and was either passed over chillers to lower the dewpoint (dry duct) or injected with steam to raise it (wet duct). The amount of aerosol removed from the distribution line was controlled by a control orifice and siphon flow rotameter. Minor adjustments to chamber concentrations were performed by changing the amount of aerosol drawn off through a HEPA-filter protected siphon flow rotameter.

AEROSOL CONCENTRATION MONITORING

Summaries of the chamber aerosol concentrations are given in Tables H3 and H4. The concentrations of methyl palmitate, methyl stearate, and methyl oleate in TRIM VX were monitored using GC/FID and compared to real-time aerosol monitor (RAM) measurements (MicroDust *pro*, Casella CEL LTD; Kempson, Bedford, England). The monitors were connected to the chambers by a sampling system designed by Battelle incorporating a valve that multiplexed each RAM to a 0 mg/m^3 chamber or the room, a HEPA-filtered room air blank, and two additional exposure chambers. The output voltage of the RAM was recorded by a program designed by Battelle (Battelle Exposure Data Acquisition and Control) to select the correct sample stream and acquire a raw voltage signal from each RAM. Equations for the calibration curves resided within the program and were used to convert the measured RAM voltages to exposure chamber concentrations. Concentration control limits within the program were compared to each measured concentration and, if limits were exceeded, an audible alarm was triggered or, in extreme cases, exposure was terminated.

Each RAM was calibrated by constructing a response curve using the measured RAM voltages (voltage readings were corrected by subtracting the RAM zero-offset voltage from measured RAM voltages) and concentrations of methyl palmitate, methyl stearate, and methyl oleate in TRIM VX that were determined by analyzing duplicate adsorbent gas sampling tubes (ORBO-52™; silica gel; Supelco, Bellafonte, PA) collected daily from the exposure chambers.

For the 3-month and 2-year studies, methyl palmitate, methyl stearate, and methyl oleate in TRIM VX were extracted from the gas sampling tubes with 2-propanol and analyzed using GC/FID by system C (Table H1) or a system similar to system C; methyl undecanoate was used as the internal standard.

The GC/FID instrument was calibrated against serially diluted standards of TRIM VX and the internal standard, methyl undecanoate. Quality control standards and a reagent blank were analyzed after calibration, after approximately every tenth sample, and at the end of the analysis to determine accuracy and calibration drift during analysis.

CHAMBER ATMOSPHERE CHARACTERIZATION

Particle size distribution in each chamber was determined prior to the start of all studies and monthly during the studies. Cascade impactor samples of TRIM VX were taken from each exposure chamber using a Mercer-style seven-stage impactor (In-Tox Products, Moriarty, NM) and the stages [22 mm glass coverslips lightly coated with silicone to prevent particle bounce for stages 1 to 7, or 25 mm Pallflex TX40HI20WW Emfab Teflon®-coated glass-fiber filters (Pell Corporation, Port Washington, NY) for stage 8] were analyzed by GD/FID using system C

(Table H1) or a similar system for methyl oleate as a marker for TRIM VX. The relative mass collected on each stage was analyzed by the CASPACT impactor analysis program developed at Battelle based on probit analysis (Hill *et al*., 1977). The resulting estimates of the mass median aerodynamic diameter and the geometric standard deviation of each set of samples are given in Tables H5 through H7. All samples were within the 1 to 3 µm range required by protocol.

Buildup and decay rates for chamber aerosol concentrations were determined with (all studies) and without (2-year studies) animals present in the chambers. At a chamber air flow rate of 15 cubic feet per minute, the theoretical value for the time to achieve 90% of the target concentration after the beginning of aerosol generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after aerosol generation was terminated (T_{10}) was approximately 9.2 minutes. For rats and mice in the 3-month studies with animals present, T_{90} values ranged from 8 to 9 minutes; T_{10} values ranged from 9 to 10 minutes. For rats and mice in the 2-year studies, T_{90} values ranged from 8 to 10 minutes without animals present and from 7 to 10 minutes with animals; T_{10} values ranged from 7 to 9 minutes without animals present and from 8 to 10 minutes with animals. T_{90} values of 10 minutes and 12 minutes were selected for the 3-month and 2-year studies, respectively.

The uniformity of aerosol concentration in the inhalation exposure chambers was measured once during the 3-month studies with animals present, once before the 2-year studies began without animals present, and approximately every 3 months during the 2-year studies with animals present. RAM measurements were taken from 12 different chamber positions. Chamber concentration uniformity was acceptable throughout the 3-month and 2-year studies.

The persistence of TRIM VX in the exposure chambers was monitored after aerosol delivery ended by monitoring the concentration in the 400 mg/m^3 chambers in the 3-month studies with animals present and in the 100 mg/m^3 chambers in the 2-year studies with and without animals present. In the 3-month studies, the concentration decreased to 1% of the starting concentration within approximately 18 minutes. In the 2-year study of male rats, the concentration decreased to 1% of the starting concentration within approximately 20 minutes with animals present and within 17 minutes without animals. In the 2-year studies of female rats and male and female mice, the concentration decreased to 1% of the starting concentration within 17 minutes with animals present and within 15 minutes without animals.

Stability studies of the test article in the generation and exposure system were performed before (2-year studies only) and during the studies by the study laboratory. Samples of the test atmosphere were taken during the first, middle, and last 2 hours of the generation day from the distribution line, 25 and 400 mg/m^3 chambers (3-month studies), and 10 and 100 mg/m^3 chambers (2-year studies); bulk samples of the test article were taken from the generator reservoir at the end of the generation day. Samples of the test atmosphere were collected using adsorbent gas sampling tubes (ORBO-52™, Supelco). Test atmosphere and generator reservoir samples were assayed for oil constituents using GC/FID by systems C (all studies) and D (2-year studies) (Table H1). Additional samples collected using bubblers filled with 2-propanol containing triethanolamine-d15 were analyzed for alkanolamines using LC/MS method A as described above. The amount of each constituent in the exposure atmosphere was calculated as a percentage of the expected amount based on concentration as determined by the on-line monitor (MicroDust *pro*, Casella CEL LTD) (3-month studies) or the amount of methyl oleate (2-year studies). Reservoir results were calculated relative to the bulk test article.

For all studies, the relative amounts of the major constituents in the exposure atmospheres and generator reservoir samples generally reflected those of the bulk test article with the exceptions of propylene glycol (in all atmosphere samples for the 3-month studies with animals present and the 2-year studies with and without animals present), diethylene glycol (in the 10 mg/m^3 atmospheres in the 2-year studies without animals present), and diethylene glycol monobutyl ether and α-terpineol (in the 10 mg/m^3 atmospheres in the 2-year studies with animals present).

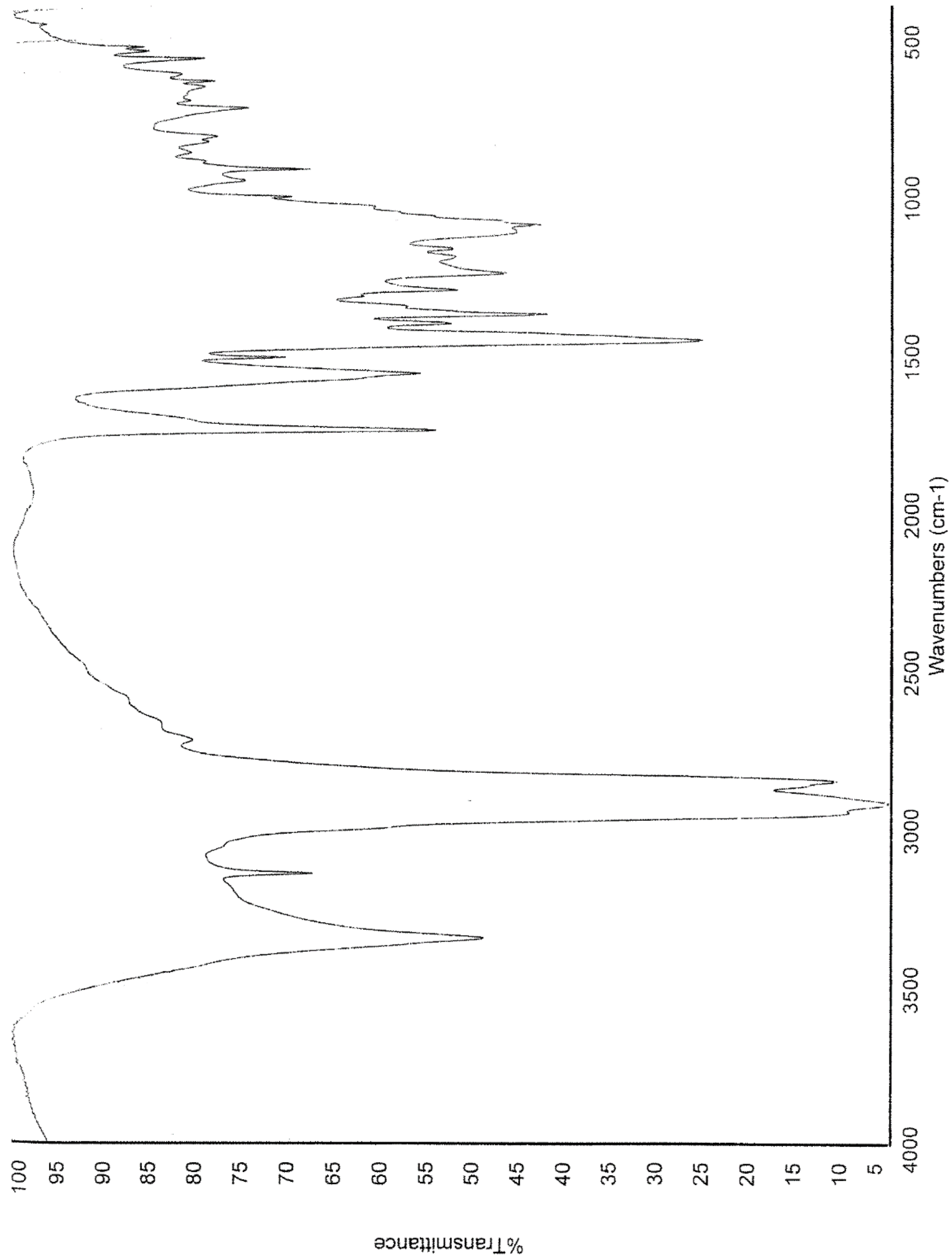

FIGURE H1
Fourier Transform Infrared Absorption Spectrum of TRIM VX

TABLE H1
Gas Chromatography Systems Used in the Inhalation Studies of TRIM VX[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	RTX®-5 Amine, 30 m × 0.32 mm, 1.0 µm film (Restek, Bellefonte, PA)	Helium at 10 psi	45° C for 1 minute, then 6° C/minute to 300° C, held for 5 minutes
System B			
Mass spectrometry	RTX®-5 Amine, 30 m × 0.25 mm, 1.0 µm film (Restek)	Helium at 10 psi	40° C for 2 minutes, then 10° C/minute to 170° C, held for 7 minutes, then 25° C/minute to 300° C, held for 10 minutes
System C			
Flame ionization	RTX®-5 Amine, 30 m × 0.32 mm, 1.0 µm film (Restek)	Helium at 10 psi	40° C for 2 minutes, then 10° C/minute to 170° C, held for 7 minutes, then 25° C/minute to 300° C, held for 10 minutes
System D			
Flame ionization	DB™-WAXETR, 30 m × 0.25 mm, 0.25 µm film (Agilent Technologies, Inc., Santa Clara, CA)	Helium at 15 psi	50° C for 2 minutes, then 25° C/minute to 280° C, held for 10 minutes

[a] The gas chromatographs and mass spectrometer were manufactured by Agilent Technologies, Inc. (Santa Clara, CA).

TABLE H2
Measured Components of the Two Lots of TRIM VX Used in the Inhalation Studies of TRIM VX[a]

Component	Lot 101607N[b]	Lot 011509N[c]
Water	7.1	6.8
Hexane extractable material	80.2	85.0
Identified organic compounds		
Triethanolamine	3.7	3.2
4-Chloro-3-methyl-phenol	3.59	2.49
Diethylene glycol	0.87	1.07
Diethylene glycol monobutyl ether	1.02	1.11
Methyl palmitate	1.18	1.20
Methyl oleate	5.65	5.81
Methyl stearate	0.89	0.93
Myristic acid	0.49	0.23
Oleic acid	3.18	1.23
Palmitic acid	1.01	0.31
Propylene glycol	0.20	0.20
α-Terpineol	0.60	0.50

[a] All values are percentages
[b] Used in the 3-month studies
[c] Used in the 2-year studies

TABLE H3
Summary of Test Material Analysis Before, During, and After the 2-Year Inhalation Studies of TRIM VX[a]

								Selected Constituents (% w/w)												
Sample Date	**Sample Description[b]**	**pH**	**Specific Gravity (g/mL)**	**Refractive Index**	**Bacteria (cfu/mL)[c]**	**Fungi (cfu/mL)[c]**	**Fourier Transform Infrared Spectroscopy[b]**	**n-Hexane Extractables**	**Triethanolamine**	**Propylene Glycol**	**Diethylene Glycol**	**DGMBE**	**α-Terpineol**	**4-Chloro-3-Methylphenol**	**Methyl Palmitate**	**Methyl Oleate**	**Methyl Stearate**	**Myristic Acid[d]**	**Palmitic Acid[d]**	**Oleic Acid[d]**
Initial Analysis																				
03/13/09	Drum 1 (top)	7.5	1.008	1.486	ND	ND	N/A	85	3.2	0.2	1.0	1.1	0.5	2.5	1.2	5.8	0.9	0.2	0.3	1.3
	Drum 1 (middle)	7.6	1.008	1.484	ND	ND		85	3.2	0.2	1.1	1.1	0.5	2.5	1.2	5.9	0.9	0.2	0.3	1.2
	Drum 1 (bottom)	7.6	1.008	1.484	ND	ND		85	3.2	0.2	1.1	1.1	0.5	2.4	1.2	5.7	0.9	0.2	0.3	1.2
Analysis Prior to Study Start																				
06/23/09 through	Drum 1	7.6	1.007	1.484	ND	ND	Consistent with	84	3.3	0.2	0.9	1.0	0.6	2.9	1.1	5.6	0.9	0.7	0.7	4.2
06/25/09	Drum 2	7.6	1.006	1.484	ND	ND	initial analysis	85	3.2	0.2	0.8	0.9	0.6	2.8	1.1	5.5	0.9	0.7	0.7	4.1
	Drum 3	7.6	1.005	1.483	ND	ND		85	3.1	0.2	0.8	0.9	0.6	2.8	1.2	5.7	0.9	0.7	0.7	3.9
	Reference[e]	7.6	1.007	1.483	ND	ND		85	3.2	0.2	0.8	1.0	0.6	2.9	1.1	5.5	0.9	0.8	0.8	4.2
Analysis During Study																				
12/01/2009 through	Drum 2	7.6	1.006	1.487	ND	ND	Consistent with	86	3.5	0.2	0.9	1.0	0.6	3.0	1.2	5.6	0.9	0.4	0.4	3.6
12/03/2009	Drum 3	7.6	1.005	1.479	ND	ND	initial analysis	85	3.0	0.2	0.8	0.9	0.6	2.9	1.2	5.6	0.9	0.4	0.4	3.4
	Drum 4	7.6	1.004	1.472	ND	ND		84	2.9	0.2	0.8	0.9	0.5	2.8	1.2	5.7	0.9	0.4	0.4	3.3
	Reference[e]	7.6	1.006	1.487	ND	ND		86	3.4	0.2	0.9	1.0	0.6	3.0	1.2	5.7	0.9	0.4	0.4	3.7
05/10/2010 through	Drum 2	7.6	1.005	1.485	ND	ND	Consistent with	84[e]	3.4	0.2	0.9	0.9	0.6	2.9	1.1	5.3	0.9	0.3	0.3	1.7
05/12/2010	Drum 3	7.6	1.005	1.484	ND	ND	initial analysis	83	3.2	0.2	0.8	0.9	0.5	2.8	1.1	5.4	0.9	0.3	0.3	1.6
	Drum 4	7.6	1.004	1.484	ND	ND		85	3.0	0.2	0.8	0.9	0.5	2.8	1.1	5.5	0.9	0.3	0.3	1.5
	Reference[e]	7.7	1.003	1.484	ND	ND		85	3.2	0.2	0.9	0.9	0.5	2.8	1.1	5.4	0.9	0.3	0.3	1.7
11/01/2010 through	Drum 3	7.5	1.007	1.484	ND	ND	Consistent with	84	3.5	0.2	0.9	1.0	0.5	2.9	1.1	5.4	0.9	0.5	0.7	3.4
11/03/2010	Drum 4	7.5	1.005	1.484	ND	ND	initial analysis	87	3.1	0.2	0.8	0.9	0.5	2.8	1.1	5.6	0.9	0.5	0.7	2.9
	Drum 5	7.5	1.005	1.484	ND	ND		85	3.0	0.2	0.8	0.9	0.5	2.8	1.2	5.6	0.9	0.5	0.6	2.9
	Reference[e]	7.5	1.005	1.483	ND	ND		84	3.3	0.2	0.9	1.0	0.5	2.9	1.1	5.5	0.9	0.5	0.7	3.4

TABLE H3
Summary of Test Material Analysis Before, During, and After the 2-Year Inhalation Studies of TRIM VX

								Selected Constituents (% w/w)												
Sample Date	**Sample Description**	**pH**	**Specific Gravity (g/mL)**	**Refractive Index**	**Bacteria (cfu/mL)**	**Fungi (cfu/mL)**	**Fourier Transform Infrared Spectroscopy[b]**	**n-Hexane Extractables**	**Triethanolamine**	**Propylene Glycol**	**Diethylene Glycol**	**DGMBE**	**α-Terpineol**	**4-Chloro-3-Methylphenol**	**Methyl Palmitate**	**Methyl Oleate**	**Methyl Stearate**	**Myristic Acid**	**Palmitic Acid**	**Oleic Acid**
Analysis During Study (continued)																				
04/15/2011	Drum 4	7.5	1.008	1.484	ND	ND	Consistent with	81	3.4	0.2	0.9	1.0	0.5	2.9	1.1	5.2	0.9	0.7	0.9	3.9
	Drum 5	7.5	1.005	1.486	ND	ND	initial analysis	85	2.9	0.2	0.8	0.9	0.5	2.8	1.1	5.5	0.9	0.7	0.9	3.5
	Reference[e]	7.5	1.004	1.485	ND	ND		85	2.9	0.2	0.8	0.9	0.5	2.9	1.1	5.4	0.9	0.7	0.9	4.1
Analysis Post Study																				
08/10/2011	Drum 5	7.6	1.008	1.485	ND	ND	Consistent with	83	3.4	0.2	0.9	0.9	0.5	2.8	1.0	4.9	0.8	0.5	0.8	3.2
	Reference[e]	7.6	1.006	1.485	ND	ND	initial analysis	84	3.1	0.2	0.9	1.0	0.5	2.9	1.1	5.1	0.8	0.5	0.8	3.4

cfu=colony forming units; ND=not detected; N/A=not applicable; DGMBE=diethylene glycol monobutyl ether

[a] Except as noted, all values reported as mean, n=3

[b] All drums tested prior to use. For initial purity analyses, three samples were taken from top, middle, and bottom of Drum 1. For subsequent analysis, three samples were taken from one location of the drum.

[c] Samples diluted 1:10 for analysis, n=1 or 2

[d] Variability in free fatty acid weight percentages between analyses (e.g., time points) was attributed to variability of the analysis method rather than a change in test material composition.

[e] Reference = a sample taken at the initial handling of the bulk test material (Drum 1) and stored at 5° C.

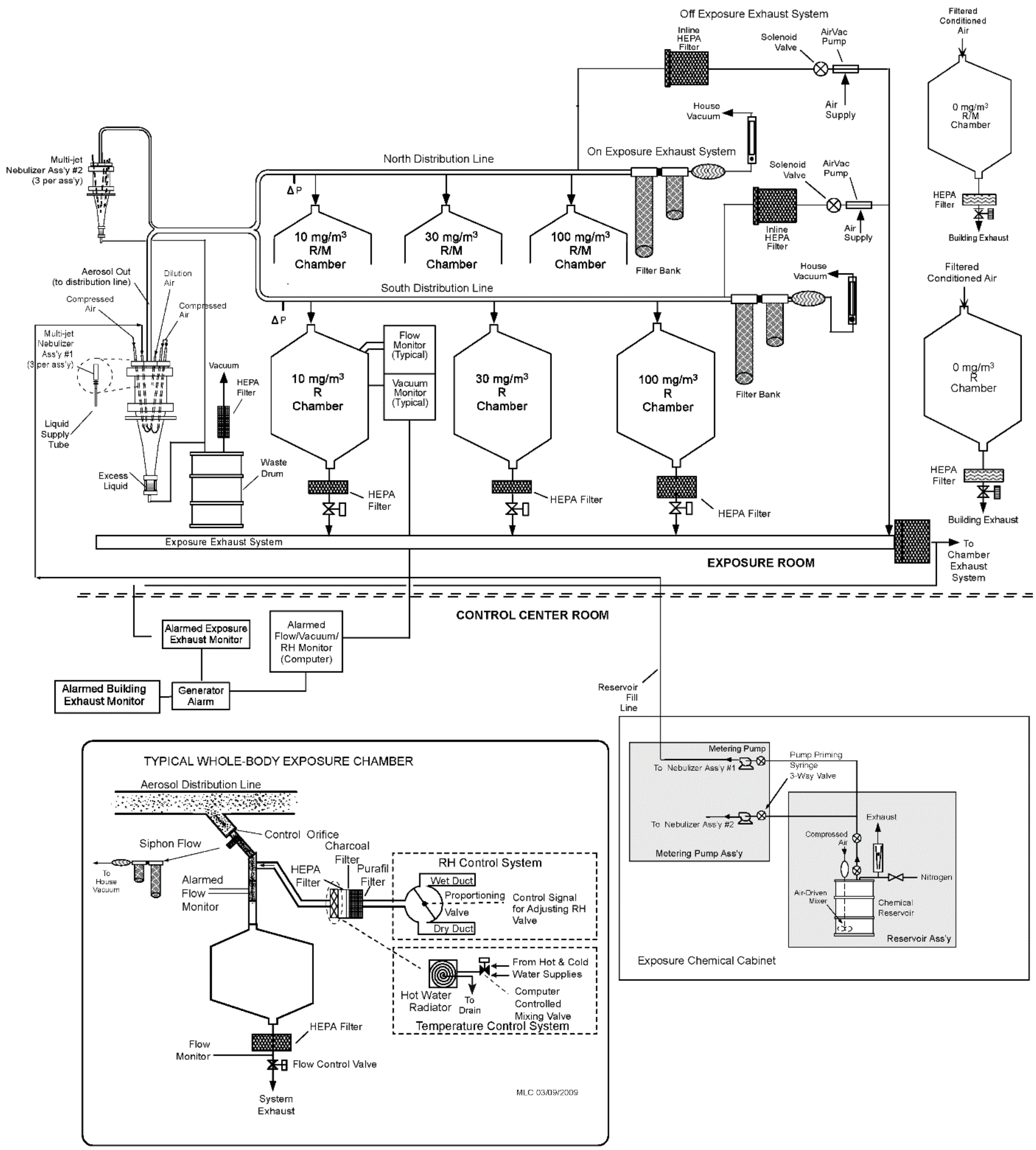

FIGURE H2
Schematic of the Aerosol Generation and Delivery System in the Inhalation Studies of TRIM VX
The exposure chamber configuration shown is for the 2-year studies.

TABLE H4
Summary of Chamber Concentrations in the 3-Month Inhalation Studies of TRIM VX

	Target Concentration (mg/m^3)	Total Number of Readings	Average Concentration[a] (mg/m^3)
Rat Chambers			
	25	684	24.9 ± 1.2
	50	681	50.1 ± 3.1
	100	684	98.7 ± 4.3
	200	681	201 ± 8.3
	400	684	380 ± 16
Mouse Chambers			
	25	704	25.0 ± 1.2
	50	701	50.2 ± 3.1
	100	704	98.8 ± 4.3
	200	701	201 ± 8.2
	400	704	381 ± 16

[a] Mean ± standard deviation

TABLE H5
Summary of Chamber Concentrations in the 2-Year Inhalation Studies of TRIM VX

	Target Concentration (mg/m^3)	Total Number of Readings	Average Concentration[a] (mg/m^3)
Male Rat Chambers			
	10	5,120	9.9 ± 0.4
	30	5,120	29.9 ± 1.1
	100	5,163	99.2 ± 2.6
Female Rat Chambers			
	10	5,184	10.0 ± 0.4
	30	5,186	30.0 ± 0.9
	100	5,140	99.3 ± 2.7
Mouse Chambers			
	10	5,175	10.0 ± 0.4
	30	5,177	30.0 ± 0.9
	100	5,133	99.3 ± 2.7

[a] Mean ± standard deviation

TABLE H6
Summary of Aerosol Size Measurements for the Rat and Mouse Exposure Chambers in the 3-Month Inhalation Studies of TRIM VX

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
July 2008	25	2.0	1.8
	50	1.9	1.9
	100	2.0	1.9
	200	2.0	1.9
	400	2.1	1.9
August 2008	25	2.1	1.8
	50	1.9	1.9
	100	1.9	1.8
	200	1.9	1.8
	400	1.9	1.8
September 2008	25	1.8	1.9
	50	1.9	1.9
	100	1.9	1.9
	200	1.8	1.9
	400	1.8	1.9
October 2008	25	1.8	1.8
	50	1.9	1.8
	100	2.0	1.9
	200	1.9	1.9
	400	2.0	1.9

TABLE H7
Summary of Aerosol Size Measurements for the Male Rat Exposure Chambers in the 2-Year Inhalation Study of TRIM VX

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
August 2009	10	2.0	1.7
	30	2.2	1.8
	100	2.0	1.8
September 2009	10	2.1	1.8
	30	2.1	1.8
	100	2.1	1.8
October 2009	10	1.9	1.7
	30	1.9	1.7
	100	2.0	1.8
November 2009	10	2.0	1.8
	30	2.0	1.8
	100	1.9	1.8
December 2009	10	2.0	1.7
	30	1.9	1.8
	100	2.1	1.8
January 2010	10	2.0	1.8
	30	1.8	1.8
	100	2.0	1.8
February 2010	10	2.0	1.8
	30	2.0	1.8
	100	2.0	1.8
March 2010	10	1.9	1.8
	30	1.9	1.8
	100	1.9	1.8
April 2010	10	2.1	1.8
	30	2.0	1.8
	100	2.0	1.8
May 2010	10	1.9	1.7
	30	1.9	1.7
	100	2.0	1.8
June 2010	10	2.0	1.8
	30	2.0	1.8
	100	2.0	1.8
July 2010	10	2.0	1.8
	30	1.9	1.8
	100	2.0	1.8
August 2010	10	2.0	1.8
	30	2.0	1.8
	100	2.0	1.8
September 2010	10	1.9	1.7
	30	1.9	1.8
	100	1.9	1.8

TABLE H7
Summary of Aerosol Size Measurements for the Male Rat Exposure Chambers in the 2-Year Inhalation Study of TRIM VX

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (μm)	Geometric Standard Deviation
October 2010	10	1.9	1.8
	30	2.0	1.8
	100	1.9	1.8
November 2010	10	2.0	1.8
	30	2.0	1.8
	100	2.0	1.8
December 2010	10	2.0	1.8
	30	2.0	1.8
	100	2.0	1.8
January 2011	10	2.0	1.8
	30	2.0	1.8
	100	2.0	1.8
February 2011	10	2.0	1.8
	30	1.9	1.8
	100	2.0	1.8
March 2011	10	2.0	1.8
	30	1.9	1.8
	100	1.9	1.7
April 2011	10	2.0	1.8
	30	2.0	1.7
	100	2.0	1.8
May 2011	10	2.0	1.7
	30	2.1	1.8
	100	2.1	1.7
June 2011	10	2.0	1.8
	30	2.0	1.8
	100	1.9	1.8
July 2011	10	1.9	1.8
	30	1.8	1.8
	100	2.0	1.8
Range	10	1.9 – 2.1	1.7 – 1.8
	30	1.8 – 2.2	1.7 – 1.8
	100	1.9 – 2.1	1.7 – 1.8

TABLE H8
Summary of Aerosol Size Measurements for the Female Rat and Male and Female Mouse Exposure Chambers in the 2-Year Inhalation Studies of TRIM VX

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (μm)	Geometric Standard Deviation
August 2009	10	2.0	1.8
	30	2.1	1.8
	100	2.1	1.8
September 2009	10	2.1	1.8
	30	2.1	1.8
	100	2.0	1.8
October 2009	10	1.8	1.7
	30	1.9	1.8
	100	2.0	1.7
November 2009	10	1.8	1.7
	30	1.9	1.8
	100	1.9	1.8
December 2009	10	1.7	1.7
	30	1.9	1.8
	100	2.0	1.8
January 2010	10	2.0	1.8
	30	1.9	1.8
	100	1.9	1.8
February 2010	10	1.9	1.7
	30	1.9	1.8
	100	2.0	1.8
March 2010	10	2.0	1.8
	30	1.9	1.8
	100	1.9	1.8
April 2010	10	2.0	1.8
	30	2.0	1.8
	100	2.0	1.8
May 2010	10	2.0	1.8
	30	1.9	1.8
	100	2.0	1.8
June 2010	10	2.0	1.8
	30	1.9	1.8
	100	1.9	1.8
July 2010	10	1.9	1.8
	30	1.9	1.8
	100	1.9	1.8
August 2010	10	2.0	1.8
	30	1.9	1.7
	100	1.9	1.7
September 2010	10	1.9	1.8
	30	1.9	1.8
	100	1.9	1.8

TABLE H8
Summary of Aerosol Size Measurements for the Female Rat and Male and Female Mouse Exposure Chambers in the 2-Year Inhalation Studies of TRIM VX

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
October 2010	10	2.0	1.8
	30	1.9	1.8
	100	1.8	1.8
November 2010	10	1.9	1.8
	30	2.0	1.8
	100	2.0	1.8
December 2010	10	2.1	1.8
	30	1.9	1.8
	100	2.1	1.8
January 2011	10	2.0	1.8
	30	1.9	1.8
	100	1.9	1.8
February 2011	10	1.9	1.7
	30	1.9	1.8
	100	1.9	1.7
March 2011	10	1.8	1.8
	30	1.9	1.7
	100	1.8	1.8
April 2011	10	2.0	1.8
	30	1.9	1.7
	100	2.0	1.7
May 2011	10	2.0	1.7
	30	1.9	1.7
	100	2.0	1.7
June 2011	10	1.9	1.8
	30	1.8	1.8
	100	1.9	1.8
July 2011	10	1.9	1.8
	30	1.9	1.8
	100	1.9	1.8
Range	10	1.7 – 2.1	1.7 – 1.8
	30	1.8 – 2.1	1.7 – 1.8
	100	1.8 – 2.1	1.7 – 1.8

APPENDIX I
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

TABLE I1 **Ingredients of NTP-2000 Rat and Mouse Ration** **174**
TABLE I2 **Vitamins and Minerals in NTP-2000 Rat and Mouse Ration** **174**
TABLE I3 **Nutrient Composition of NTP-2000 Rat and Mouse Ration** **175**
TABLE I4 **Contaminant Levels in NTP-2000 Rat and Mouse Ration** **176**

TABLE I1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

TABLE I2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

TABLE I3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.6 ± 0.35	14.1 – 15.4	31
Crude fat (% by weight)	8.4 ± 0.27	7.7 – 9.0	31
Crude fiber (% by weight)	9.4 ± 0.97	7.1 – 11.8	31
Ash (% by weight)	5.0 ± 0.18	4.7 – 5.4	31
Amino Acids (% of total diet)			
Arginine	0.789 ± 0.071	0.67 – 0.97	24
Cystine	0.219 ± 0.023	0.15 – 0.25	24
Glycine	0.700 ± 0.039	0.62 – 0.80	24
Histidine	0.349 ± 0.075	0.27 – 0.68	24
Isoleucine	0.546 ± 0.042	0.43 – 0.66	24
Leucine	1.095 ± 0.064	0.96 – 1.24	24
Lysine	0.703 ± 0.114	0.31 – 0.86	24
Methionine	0.409 ± 0.044	0.26 – 0.49	24
Phenylalanine	0.628 ± 0.038	0.54 – 0.72	24
Threonine	0.507 ± 0.041	0.43 – 0.61	24
Tryptophan	0.151 ± 0.028	0.11 – 0.20	24
Tyrosine	0.409 ± 0.064	0.28 – 0.54	24
Valine	0.664 ± 0.042	0.55 – 0.73	24
Essential Fatty Acids (% of total diet)			
Linoleic	3.96 ± 0.250	3.49 – 4.55	24
Linolenic	0.30 ± 0.031	0.21 – 0.35	24
Vitamins			
Vitamin A (IU/kg)	3,776 ± 66	2,110 – 5,330	31
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	79.7 ± 21.28	27.0 – 124.0	24
Thiamine (ppm)[b]	7.7 ± 1.50	5.3 – 12.3	31
Riboflavin (ppm)	7.90 ± 2.96	4.20 – 17.50	24
Niacin (ppm)	78.9 ± 8.86	66.4 – 98.2	24
Pantothenic acid (ppm)	27.0 ± 12.08	17.4 – 81.0	24
Pyridoxine (ppm)[b]	9.58 ± 1.91	6.44 – 13.7	24
Folic acid (ppm)	1.60 ± 0.47	1.15 – 3.27	24
Biotin (ppm)	0.32 ± 0.10	0.20 – 0.704	24
Vitamin B_{12} (ppb)	52.8 ± 38.0	18.3 – 174.0	24
Choline (ppm)[b]	2,733 ± 608	1,160 – 3,790	24
Minerals			
Calcium (%)	0.898 ± 0.047	0.810 – 0.994	31
Phosphorus (%)	0.568 ± 0.054	0.504 – 0.822	31
Potassium (%)	0.669 ± 0.031	0.626 – 0.733	24
Chloride (%)	0.384 ± 0.038	0.300 – 0.474	24
Sodium (%)	0.193 ± 0.024	0.160 – 0.283	24
Magnesium (%)	0.217 ± 0.059	0.185 – 0.490	24
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	14
Iron (ppm)	188 ± 38.3	135 – 311	24
Manganese (ppm)	51.2 ± 9.99	21.0 – 73.1	24
Zinc (ppm)	59.02 ± 27.84	43.3 – 184	24
Copper (ppm)	7.28 ± 2.635	3.21 – 16.3	24
Iodine (ppm)	0.504 ± 0.197	0.158 – 0.972	24
Chromium (ppm)	0.683 ± 0.270	0.330 – 1.380	23
Cobalt (ppm)	0.242 ± 0.162	0.094 – 0.864	22

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE I4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.25 ± 0.047	0.17 – 0.42	31
Cadmium (ppm)	0.06 ± 0.012	0.04 – 0.10	31
Lead (ppm)	0.11 ± 0.015	0.06 – 0.89	31
Mercury (ppm)	<0.02		31
Selenium (ppm)	0.19 ± 0.048	0.09 – 0.34	31
Aflatoxins (ppb)	<5.00		31
Nitrate nitrogen (ppm)[c]	18.91 ± 8.42	10.0 – 42.3	31
Nitrite nitrogen (ppm)[c]	<0.61		31
BHA (ppm)[d]	<1.0		31
BHT (ppm)[d]	1.10 ± 0.366	1.0 – 3.04	31
Aerobic plate count (CFU/g)	15.48 ± 28.7	10 – 170	31
Coliform (MPN/g)	3.0 ± 0.0	3.0	31
Escherichia coli (MPN/g)	<10		31
Salmonella (MPN/g)	Negative		26
Total nitrosoamines (ppb)[e]	9.2 ± 4.54	2.0 – 19.5	31
N-Nitrosodimethylamine (ppb)[e]	2.8 ± 2.15	1.0 – 9.6	31
N-Nitrosopyrrolidine (ppb)[e]	6.6 ± 3.72	1.0 – 15.0	31
Pesticides (ppm)			
α-BHC	<0.01		31
β-BHC	<0.02		31
γ-BHC	<0.01		31
δ-BHC	<0.01		31
Heptachlor	<0.01		31
Aldrin	<0.01		31
Heptachlor epoxide	<0.01		31
DDE	<0.01		31
DDD	<0.01		31
DDT	<0.01		31
HCB	<0.01		31
Mirex	<0.01		31
Methoxychlor	<0.05		31
Dieldrin	<0.01		31
Endrin	<0.01		31
Telodrin	<0.01		31
Chlordane	<0.05		31
Toxaphene	<0.10		31
Estimated PCBs	<0.20		31
Ronnel	<0.01		31
Ethion	<0.02		31
Trithion	<0.05		31
Diazinon	<0.10		31
Methyl chlorpyrifos	0.106 ± 0.116	0.020 – 0.553	31
Methyl parathion	<0.02		31
Ethyl parathion	<0.02		31
Malathion	0.125 ± 0.099	0.020 – 0.400	31
Endosulfan I	<0.01		31
Endosulfan II	<0.01		31
Endosulfan sulfate	<0.03		31

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride

[b] For values less than the limit of detection, the detection limit is given as the mean.

[c] Sources of contamination: alfalfa, grains, and fish meal

[d] Sources of contamination: soy oil and fish meal

[e] All values were corrected for percent recovery.

APPENDIX J
SENTINEL ANIMAL PROGRAM

METHODS **178**
RESULTS **180**

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or dosed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

Blood samples were collected from each animal and allowed to clot and the serum was separated. Additionally, fecal samples were collected and tested for *Helicobacter* species. All samples were processed appropriately with serology testing performed in-house or sent to Research Animal Diagnostic Laboratory, University of Missouri, Columbia, MO, for determination of the presence of pathogens. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Blood was collected from five animals per sex per time point.

Method and Test	Time of Collection
RATS	
3-Month Study	
ELISA	
Mycoplasma pulmonis	1 week
Pneumonia virus of mice (PVM)	1 week
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	1 week
Rat parvovirus	1 week
Sendai	1 week
Multiplex Fluorescent Immunoassay	
Kilham rat virus (KRV)	Study termination
M. pulmonis	Study termination
Parvo NS-1	Study termination
PVM	Study termination
RCV/SDA	Study termination
Rat minute virus (RMV)	Study termination
Rat parvovirus (RPV)	Study termination
Rat theilovirus (RTV)	Study termination
Sendai	Study termination
Theiler’s murine encephalomyelitis virus (TMEV)	Study termination
Toolan’s H-1 virus	Study termination

Method and Test	Time of Collection
RATS (continued)	
2-Year Study	
Multiplex Fluorescent Immunoassay	
KRV	1 week; 6, 12, and 18 months, study termination
M. pulmonis	1 week; 6, 12, and 18 months, study termination
Parvo NS-1	1 week; 6, 12, and 18 months, study termination
PVM	1 week; 6, 12, and 18 months, study termination
RCV/SDA	1 week; 6, 12, and 18 months, study termination
RMV	1 week; 6, 12, and 18 months, study termination
RPV	1 week; 6, 12, and 18 months, study termination
RTV	1 week; 6, 12, and 18 months, study termination
Sendai	1 week; 6, 12, and 18 months, study termination
TMEV	1 week; 6, 12, and 18 months, study termination
Toolan's H-1 virus	1 week; 6, 12, and 18 months, study termination
Immunofluorescence Assay	
M. pulmonis	12 and 18 months
RTV	Study termination
MICE	
3-Month Study	
ELISA	
Mouse hepatitis virus (MHV)	1 week
Mouse parvovirus (MPV)	1 week
M. pulmonis	1 week
PVM	1 week
Sendai	1 week
Theiler's murine encephalomyelitis virus – mouse poliovirus, strain GDVII (TMEV GDVII)	1 week
Multiplex Fluorescent Immunoassay	
Ectromelia virus	Study termination
Epizootic diarrhea of infant mice (EDIM)	Study termination
Lymphocytic choriomeningitis virus (LCMV)	Study termination
M. pulmonis	Study termination
MHV	Study termination
Mouse norovirus (MNV)	Study termination
MPV	Study termination
Minute virus of mice (MVM)	Study termination
Parvo NS-1	Study termination
PVM	Study termination
Reovirus	Study termination
TMEV GDVII	Study termination
Sendai	Study termination

Method and Test	Time of Collection
MICE (continued)	
2-Year Study	
Multiplex Fluorescent Immunoassay	
Ectromelia virus	1 week; 6, 12, and 18 months, study termination
EDIM	1 week; 6, 12, and 18 months, study termination
LCMV	1 week; 6, 12, and 18 months, study termination
M. pulmonis	1 week; 6, 12, and 18 months, study termination
MHV	1 week; 6, 12, and 18 months, study termination
MNV	1 week; 6, 12, and 18 months, study termination
Parvo NS-1	1 week; 6, 12, and 18 months, study termination
MPV	1 week; 6, 12, and 18 months, study termination
MVM	1 week; 6, 12, and 18 months, study termination
PVM	1 week; 6, 12, and 18 months, study termination
Reovirus	1 week; 6, 12, and 18 months, study termination
TMEV GDVII	1 week; 6, 12, and 18 months, study termination
Sendai	1 week; 6, 12, and 18 months, study termination
Polymerase Chain Reaction	
Helicobacter species	18 months

RESULTS

All test results were negative.

APPENDIX K
SUMMARY OF PEER REVIEW PANEL COMMENTS

DRAFT NTP TECHNICAL REPORT ON TRIM® VX (TR 591) **182**

SUMMARY OF PEER REVIEW PANEL COMMENTS

DRAFT NTP TECHNICAL REPORT ON TRIM® VX (TR 591)

Presentation
NTP study scientist Dr. K.R. Ryan briefed the panel on the draft NTP technical report on TRIM® VX. TRIM® VX is a water-soluble metalworking fluid (MWF) in the class known as soluble oils. Due to their high production volume, the large number of occupationally exposed workers, and the lack of carcinogenicity and toxicology data, the National Institute for Occupational Safety and Health nominated MWFs for NTP study. TRIM® VX was one of four MWFs selected for study from the original slate of 30.

NTP conducted 3-month and 2-year whole body inhalation exposure studies in male and female Wistar Han rats and male and female B6C3F1/N mice.

The 3-month study showed that the major target of TRIM® VX exposure was the respiratory tract, with similar toxicity in both sexes and species. Lung fibrosis was also seen, which was a distinct finding in NTP comparison studies with MWFs. No effects were seen on overall survival, clinical observations, or body weights in either sex or species.

Based on the 2-year studies, the draft NTP report's conclusions on TRIM® VX were:

Male Wistar Han rats
- *Equivocal evidence of carcinogenic activity*
  - Combined occurrences of alveolar/bronchiolar adenoma or carcinoma of the lung

Female Wistar Han rats
- *Equivocal evidence of carcinogenic activity*
  - Occurrences of alveolar/bronchiolar adenoma of the lung

Male B6C3F1/N mice
- *Clear evidence of carcinogenic activity*
  - Increased combined incidences of alveolar/bronchiolar adenoma or carcinoma of the lung

Female B6C3F1/N mice
- *Clear evidence of carcinogenic activity*
  - Increased combined incidences of alveolar/bronchiolar adenoma or carcinoma (primarily carcinoma) of the lung

Exposure to TRIM® VX resulted in increased incidences of nonneoplastic lesions of the lung, nose, and larynx of male and female rats and mice; the bronchial lymph node in male and female rats and male mice, and the mediastinal lymph node in male and female rats.

Questions for Clarification
Dr. Sabo-Attwood asked Dr. Ryan to comment on the bacterial fungal growth assays performed, and whether they also included an endotoxin assay. Dr. Ryan said they had not done an endotoxin assay. An assessment for bacterial and fungal growth was done, and no evidence of bacterial or fungal growth was detected throughout the studies.

Dr. Brock questioned the stability assessments performed on the test article. He asked for clarification on Dr. Ryan's presentation to "data compared to frozen reference sample upon receipt of the material." Dr. Ryan replied that when the test material was received, aliquots were taken out and frozen, so that current data could be compared to the reference samples at any point during the study to see if there was any degradation over time. Dr. Brock noted that method assumed that the frozen samples would not also degrade. Dr. Ryan said no evidence of degradation was seen. Dr. S. Waidyanatha, chemistry group leader in the Program Operations Branch of NTP, described the procedures that had been conducted to assess stability and clarified that the test article was stored at 5° C. Dr. Brock said he found the description in the report confusing.

Public Comments

Dr. Mirsalis noted receipt and distribution to the panel of written comments from Ms. Holly Alfano of the Independent Lubricant Manufacturers Association (ILMA) and Dr. Steven Florio of Master Chemical Corporation (MCC). Dr. Mirsalis then recognized an oral public commenter, Dr. Franklin Mirer of the CUNY School of Public Health, who spoke on his own behalf by telephone.

Dr. Mirer described the history of the MWFs project, which began with a petition to the NTP from the United Auto Workers. He noted that the respiratory effects of MWFs are as important to public health as carcinogenic effects. He noted the contrast between fresh fluids and in-use, contaminated fluids. He said that the respiratory effects of in-use MWFs of all types are generally accepted, with the dispute being whether they stem from microbial contamination or whether the fluids themselves have toxic potential. He cited several previous studies from the literature. He asked the NTP to publish complete analyses of all nine test articles, especially the four articles subjected to 90-day testing. He noted that MCC has already discontinued TRIM® VX. He pointed out that sulfonate had not appeared as a component of TRIM® VX in NTP analysis. He stated that lung tumors in male rats should be "some evidence," not "equivocal evidence." He agreed with the "clear evidence" conclusion for lung tumors in mice of both genders.

Next, Dr. Mirsalis acknowledged a series of four public commenters. Dr. Walden Dalbey of DalbeyTox, LLC, and Dr. John Howell of GHS Resources, Inc., spoke on behalf of ILMA. Dr. Patricia Beattie of SciVera, LLC, and Dr. Steven Florio of Master Chemical Corporation spoke on behalf of MCC.

Dr. Dalbey asked the NTP to clarify the rationale for the selection of TRIM® VX. He questioned the handling of TRIM® VX samples and asked why the material had not been diluted with water, as is common in the workplace. He asked for the rationale and validation of the methods used to determine particle size and monitor total aerosol concentration. He noted a discrepancy in the NTP's analysis of mineral oil content in TRIM® VX compared with the Material Safety Data Sheet. He noted other issues with the NTP's analyses of TRIM® VX, including the reported pH of the test article. He asked for a more explicit statement on the lack of systemic effects than what is currently in the draft report. He recommended the NTP consider a different mode of action than what is stated in the report.

Dr. Beattie provided details about TRIM® VX and the history of its toxicity testing. She noted that the tested TRIM® VX concentrate would become alkaline when mixed with the moisture in the respiratory tract, likely causing the irritation, inflammation, and tumors formed at the site of contact. She said that the negative results in genotoxicity tests, the lack of systemic toxicity or tumors, and tumors only seen at the site of contact at the highest dose, all suggest a non-genotoxic mechanism. She noted that she made a request to the NTP for more detailed chemical characterization and stability analytical information, and NIEHS denied the release of the additional analytical information.

Dr. Florio provided further background information about TRIM® VX and listed several analytical concerns related to the NTP study, particularly the chemical degradation and analysis of the test article. He noted that the test article used in the study had aged outside of its recommended 1-year shelf life, affecting its stability. He concluded that the two lots of material tested were not chemically equivalent to the TRIM® VX produced and marketed by MCC.

Dr. Howell discussed the NTP selection process for MWFs, with which ILMA had cooperated. He asserted that the NTP should find the TRIM® VX study inadequate because of significant issues regarding the characterization of the test article, such as the potential for bacterial and fungal growth, variations in the characterization of the compound, and use of the product well beyond its stated shelf life. He noted that TRIM® VX is a unique formulation and that according to Occupational Safety and Health Administration regulations, the study results cannot be extended to other MWFs.

Peer Reviewer Comments

Dr. Elwell, the first reviewer, stated that overall the report was well written and the results supported the conclusions. Regarding the findings, he cited Table 9 in the report, and asked why some of the nonneoplastic results (nasal polyps) listed in the summary table in the appendix had not been discussed in the body of the report. He asked whether the cystic keratinizing epithelioma (CKE) seen in a high-dose female had been considered to be part of the equivocal evidence. He recommended adding discussion in the report about the occurrence of multiple adenomas in rats and mice. He asked for clarification of the sentence on page 62, "Alveolar/bronchiolar neoplasms were morphologically typical of those that occur spontaneously." He agreed in principal with the conclusions; however, he suggested considering CKE as part of the equivocal evidence and asked for further information about nasal tumors in rats for possible inclusion in the report.

Dr. Ryan said that the nasal polyps appeared to be more inflammatory than neoplastic, and were deemed to not warrant inclusion in the report's text. NTP study pathologist Dr. R.A. Herbert agreed with Dr. Ryan's statement. Regarding the CKE, Dr. Ryan said that the corresponding text in the report could be clarified, and noted that since it was a single incidence, it was not considered part of the call. Regarding the multiple adenomas in the rats and mice, she said additional discussion would be added to report. Dr. Herbert explained that the bronchial adenoma had been listed as a single finding and was not included in the call. Dr. Ryan said further text changes to the report would be considered to provide clarification.

Dr. Brock, the second reviewer, said that the report was generally well-written. He noted that because TRIM® VX is a mixture and the specific chemical composition has not been revealed, the results from the current TRIM® VX studies make it difficult to determine the potential hazard associated with other soluble metal working fluids. He suggested that the authors insert an appendix or other reference for the chemical composition of other soluble MWFs to allow subsequent use of the toxicological data in the report for comparison to other mixtures. He noted the discrepancy between the NTP's stated chemical composition for TRIM® VX and the manufacturer's listing. He stated the high exposure concentration used in the 2-year studies was too high, and that an exposure concentration of 50 mg/m^3 would have been sufficient. Thus, he asked for more description of exposure concentration selection in the discussion section of the report. He proceeded to provide specific recommendations for each section of the report. Notably, he asked for clarification on the methods used to test the stability of the test article and validate aerosol chamber concentrations. He asked for an explanation on what was unique about the occurrence of lung fibrosis.

Regarding Dr. Brock's comment on the selection of TRIM® VX, Dr. Ryan said that TRIM® VX was selected as an example of a soluble oil and not to reflect all soluble oils. She said this would be clarified in the report. As to the exposure concentration selection rationale, she said the aim was to design a study to allow cross-species comparison; thus, the concentration needed to challenge both rats and mice. An exposure concentration of 50 mg/m^3 would have been sufficient in rats; however, a higher concentration was needed to challenge the mice. Thus, 100 mg/m^3 was chosen as an exposure concentration to not limit survival or have overt toxicity. There was also an aim to compare the TRIM® VX study to the prior CIMSTAR study, which had similar exposure concentrations. Dr. Brock said that although he appreciated the complexity of 2-year bioassays, using the same exposure concentration in rats and mice because it is easier is not a good answer. He renewed his request for a more robust exposure concentration justification. Dr. Ryan responded to several of Dr. Brock's specific suggestions. Regarding test article stability, she said that it was evaluated with a qualified method described in the appendix, and that there was no evidence of degradation in the bulk test article. She stated that the analysis of the chamber concentration was based on the quantitative assessment of three TRIM® VX components as mentioned in the appendix. She said additional text could be added to the report to further describe the methods for test article stability and validating aerosol chamber concentrations. She noted that data regarding clinical signs of toxicity were available on a website, which would be added to the report. Regarding the lung fibrosis, it was not unique on its own; however, it was interesting that lung fibrosis was found in the TRIM® VX studies in both species and this fibrosis was not seen in NTP studies of other MWFs.

Dr. Sabo-Attwood, the third reviewer, said the report represented a scientifically sound study and was well written. She asked for more clarity about the fibrotic lesions. Like Dr. Brock, she thought TRIM® VX was representative of the larger class of water-soluble MWFs based on the text in the draft report. She recommended edits to the text to avoid that confusion. TRIM® VX is a complex mixture, so it is unclear how the component chemicals are distributed *in vivo*. Based on the description provided, she anticipated that the constituents would be highly soluble and would not be the particulates in the aerosols that would be consistent with fibrotic lesions associated with particulate exposure. She noted the fibrotic lesions are described in the report as those commonly seen with irritation. She asked for clarification on what the irritation was and whether it was a distinguishing lesion for this particular material, especially compared to other MWFs that were tested. She noted tissues were stained with Oil-Red-O to indicate the presence of TRIM® VX in immune cells, inflammatory lesions, and hyperplastic alveolar epithelium; however, the increase in staining could also represent phospholipid inclusions due to disruption of lung surfactant or result from cell death, perhaps of alveolar cells (Epping *et al*., 2011). She suggested additions to the report because there are limited data available regarding metabolism and clearance of TRIM® VX. She also asked for explanation for the use of Oil-Red-O stain in rats and not in mice. She asked about endotoxin testing, and whether the inflammatory responses that were seen were indicative of exposures to endotoxins or other pathogens.

Dr. Ryan would consider adding text to further describe the fibrotic lesions in the report; however, the studies were not designed to directly link particulates or components of TRIM® VX with the fibrotic lesions. The fibrotic lesions were not indicative of the precancerous lesions. Dr. Herbert said that the fibrotic changes were not considered a part of the continuum from preneoplastic change to the tumors that were seen in the lung, which were mainly alveolar/bronchiolar carcinomas. He explained that Oil-Red-O staining had not been done in mice because the entire mouse lung tissue was embedded for preparation of histological slides; therefore, there was no residual wet lung tissue that could be used to perform Oil-Red-O staining. He said that the stain had been used because although TRIM® VX is soluble, the lesions were morphologically similar to those observed with inhalation exposure to a particulate. Thus, there was an interest in seeing if there was an appreciable amount of oil left in the lung that was causing a foreign body reaction. Residue from oil was found in the tissues. Dr. Sabo-Attwood asked if one could distinguish it from material identified as TRIM® VX and phospholipid damage, which would show up from staining. Dr. Herbert said he would add statements to the report to reflect Dr. Sabo-Attwood's comments. Dr. Brock asked if the tissue had been formalin-fixed, which would remove all lipids. Dr. Herbert said the lipid was not removed by formalin fixation of the lungs and would only be removed if the tissue were subjected to histological processing. Thus, the wet tissue would still have oil present in the lungs. Dr. Ryan noted that endotoxin was not measured in the study; however, there was assessment of bacterial and fungal growth at several study time points. There was no evidence of bacterial or fungal growth.

Dr. Sabo-Attwood asked if there was anything indicative in the inflammatory response that was relevant to an endotoxin-type exposure. Dr. Herbert explained that with so many inflammatory changes, it was not possible to delineate whether they were due to an endotoxin or exposure and direct contact with the chemicals.

Panel Discussion and Vote

Dr. Singal asked about necrotic lesions that were attributed to increased exudative pressure, and inquired about other characteristics aside from the necrotic changes that would suggest the potential for increased barotrauma.
Dr. Herbert said there was an accumulation of exudate in the ventral portions of the nasal cavity, which was severe in some cases and most likely resulted in pressure degeneration, necrosis, and ultimately rupture and perforation of the nasal septa.

Dr. Pinkerton asked if the lung fibrosis was thought to lead to greater potential for carcinogenesis. Dr. Pinkerton asked if there was any evidence that greater fibrosis was associated with greater propensity to see tumors.
Dr. Herbert replied that could not be stated with any certainty. There are some inhalation studies with particulates where fibrosis is observed without this type of scar tumor.

Dr. Mirsalis commented on the selection of TRIM® VX. He suggested making the following points clear in the introduction: TRIM® VX is an example of a MWF, relatively small volume of it was in use, and it has since been discontinued. He noted that wider conclusions about soluble MWFs should not and could not be drawn based on this study, which stands on its own. Dr. J.R. Bucher confirmed Dr. Mirsalis' statement, adding that there should be an idea from the discussions about how difficult it was to sort through all of the different products on the market and determine their identifiable components versus trade secret components. It was difficult to select materials for 2-year study that would give some indication of whether some of the effects that were seen in the MWFs could be attributed to materials that were not contaminated with bacteria during the course of their use. Due to the complexity of the field, the materials chosen are not representative, but are individual materials.

Regarding the question of the NTP's chemical analysis of the materials, Dr. Mirsalis said that the information should be included in the final report, as it should be readily available. Regarding the earlier comment that the study should be considered inadequate because the material was not actually tested, he said that the study is an adequate study. One may argue the relevance of the study; however, it meets the requirements for an adequate study.

With no further discussion and no requests for edits to the conclusions, Dr. Mirsalis called for a motion on the conclusions. Dr. Elwell moved that the conclusions be accepted as written. Dr. Pinkerton seconded.

Dr. Brock asked for further clarification on the issue of the fibrosis and the carcinomas seen in the study.
Dr. Herbert elaborated that in terms of the neoplasms seen in the study, fibrosis was not seen as a prerequisite.

Dr. Mirsalis called for a vote on the motion. The panel voted 5-0 to accept the conclusions as written. Dr. Pino was recused and not present during the peer review of the draft NTP Technical Report on TRIM® VX.